Studies of Fish Spoilage

VIII. Volatile Acid of Cod Muscle Press Juice

By V. K. Collins
Atlantic Fisheries Experimental Station

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ABSTRACT

Acetic acid has been identified as the only volatile acid produced in spoiling cod muscle press juice. Lactic acid present and that formed from some unknown precursor is oxidized to acetic acid and carbon dioxide. The significance of these results is discussed.

The development of volatile acid during storage of fish has been used as a criterion of the state of freshness (Hillig 1939, Hillig and Clark 1938). Malin (1939) used the acid number of an ether extract for the same purpose. In a study of the oxidation of lactic acid by washed suspensions of *Achromobacter* capable of reducing trimethylamine oxide, Watson (1939) postulated the reaction,

$$CH_3.CHOH.COOH + 2(CH_3)_3.NO + H_2O \rightarrow CH_3.COOH + 2(CH_3)_3.N + CO_2 + 2H_2O.$$

All substances believed to take part in the reaction, and all substances believed produced were found to agree with the theoretical values, with the exception of water and of acetic acid which was not demonstrated. It is necessary, therefore, to determine whether acetic acid can be identified in spoiling fish, and whether the simple reaction outlined by Watson plays an important role in the metabolism of the bacteria concerned with this spoilage.

In previous papers from this laboratory, fish spoilage has been considered to be any change which is known to render the fish unacceptable or objectionable to any appreciable proportion of consumers. Trimethylamine is objectionable to many people, and it is formed in sea fishes very early in the post-mortem changes; for this paper spoiled or spoiling fish are those in which trimethylamine is developing rapidly, or has reached a high value.

METHODS

The preparation of the substrate, the conditions at the start of the storage period and the terminology are identical with those described previously (Beatty and Collins 1939).

Analytical Methods

Lactic acid was measured by the method of Friedemann and Graeser (1933), after the removal of proteins by the mercuric sulphate-barium carbonate technique of West, Scharles and Peterson (1929).

Volatile acid was separated and identified by the method of Friedemann (1938).

Carbon dioxide was measured by the Van Slyke volumetric method as modified by Danielson and Hastings (1939). This modification avoids the introduction of the sample into the chamber and subsequent tedious cleaning.

Recovery from known solutions was over 99 per cent in all three procedures.

Trimethylamine was determined by steam-vacuum distillation in the Beatty-Gibbons modification of the Parnas-Mozolowski still (Beatty and Gibbons 1937).

Trimethylamine oxide was determined by reduction with Devarda's alloy and hydrochloric acid as outlined by Beatty (1938).

Identification of Volatile Acid

Although Watson (1939) postulated the production of acetic acid from lactic, his work was done on pure substrates and it is to be expected that other volatile acids might be produced from press juice, particularly in view of Hillig's work (1939). In order to identify the acid or acids volatile in steam a number of preliminary experiments were carried out, fractionating the distillate from the magnesium sulphate into six fractions (modified Duclaux method) and determining the rate of distillation from the titre of each, also the distribution constant between ether and water of each. In no case did either of these criteria vary by more than experimental error from those obtained by using pure acetic acid. It was therefore concluded that no other volatile acid was formed, and in subsequent experiments the distillate was not fractionated, but distilled over into a volumetric flask, made to volume, one aliquot titrated and another used to determine the distribution constant. Lactic acid, to the extent present in the press juice, was found not to interfere. No evidence for the production of unsaturated volatile acids was found.

LACTIC ACID DEGRADATION AND ACETIC ACID PRODUCTION

The relationship of lactic acid breakdown to acetic acid production in the absence of air is shown in figures 1 and 3 and in the presence of air in figures 2 and 4. In every case the acetic acid produced is greater than the lactic acid disappearing, the discrepancy being more marked in figures 1 and 2. But this disagreement with Watson's reaction may be only apparent. Known sugars that might be precursors of acetic acid can account for only a small part of the difference. Especially in figures 1 and 2 the lactic acid is shown to increase for the first day or two. This increase in lactic acid noted previously by Beatty and Collins (1939) is sometimes very marked. It will be noted as well that after two days, the curve of acetic acid production is almost the mirror image of that of lactic acid destruction. It is logical to assume that since part of the lactic acid is produced from some precursor and is being oxidized at the same time to acetic, the total lactic acid may be sufficiently large to account for all or a very large part of the acetic acid produced.

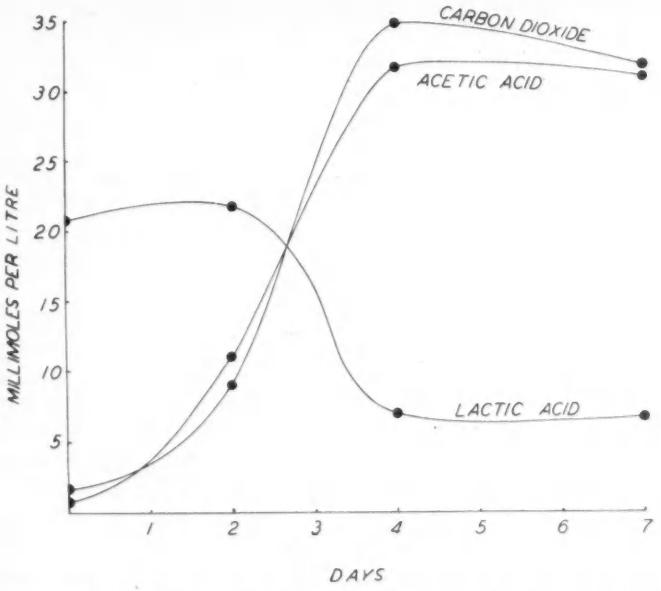

FIGURE 1. Changes in lactic acid, acetic acid, and carbon dioxide in cod muscle press juice stored at 5°C. in the absence of air.

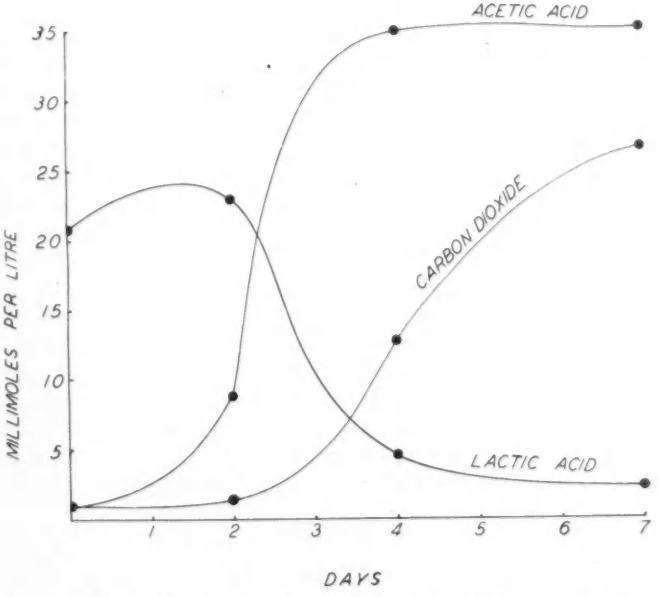

FIGURE 2. Changes in lactic acid, acetic acid and carbon dioxide in cod muscle press juice stored at 5°C. in the presence of air.

CARBON DIOXIDE PRODUCTION

In pure cultures and under anaerobic conditions Watson (1939) found the increase in carbon dioxide to be equivalent to the decrease in lactic acid, and concluded from his equation that the increase in acetic acid, which was not measured, was also equivalent.

In press juice in the absence of air (figure 1) the production of carbon dioxide more closely parallels the increase of acetic acid than the decrease in lactic acid. This fact lends weight to the assumption that acetic acid is produced from lactic acid, which in turn is produced in part from some hitherto unrecognizable precursor, the lactic acid being formed and broken down simultaneously.

In the reaction (page 197) outlined by Watson to explain what is probably the most important role of trimethylamine in the respiration of certain of the facultative anaerobes concerned with fish spoilage, values for lactic acid, trimethylamine oxide, trimethylamine, and carbon dioxide all agreed within experimental error. Since the values for acetic acid and carbon dioxide in figure 1 agree closely, and since at least sufficient acetic acid is produced to account for all lactic acid oxidized, one can conclude that the above reaction actually does occur.

In the presence of air (figure 2) the relationship is less clear. The production of carbon dioxide is slower than either the fall in lactic acid or the rise in acetic acid. It is possible that the carbon dioxide produced becomes in itself a hydrogen acceptor; no carbon dioxide would be lost at the hydrogen-ion concentration met with in muscle press juice.

RELATION TO TRIMETHYLAMINE OXIDE

Watson's equation requires the production of two moles of trimethylamine from the oxide for each mole of lactic acid oxidized. As in the case of the carbon dioxide, the agreement between acetic acid produced and trimethylamine formed (figures 3 and 4) is very much better than between the lactic acid disappearing and trimethylamine formed. Even so, more trimethylamine is formed than can be accounted for by production of acetic acid at the ratio of two moles of the former to one of the latter. Trimethylamine oxide perhaps acts as a hydrogen acceptor for other minor oxidations as well as that of lactic acid, sugar, for example.

In the absence of air, trimethylamine production ceases before all trimethylamine oxide is used up even when a relatively large proportion of lactic acid is left. This fact has been demonstrated in a large number of previous experiments. At this point the progress of spoilage practically ceases. In the presence of air all the trimethylamine oxide was used up. As shown previously (Beatty and Collins 1939), in the presence of air other constituents are involved and, as would be expected, spoilage continues after the trimethylamine is exhausted.

DISCUSSION

Acetic acid could be used as a guide to the state of preservation but the determination is too slow to be used as a routine method. It would serve to verify other methods in doubtful cases, and as a criterion for the reliability of

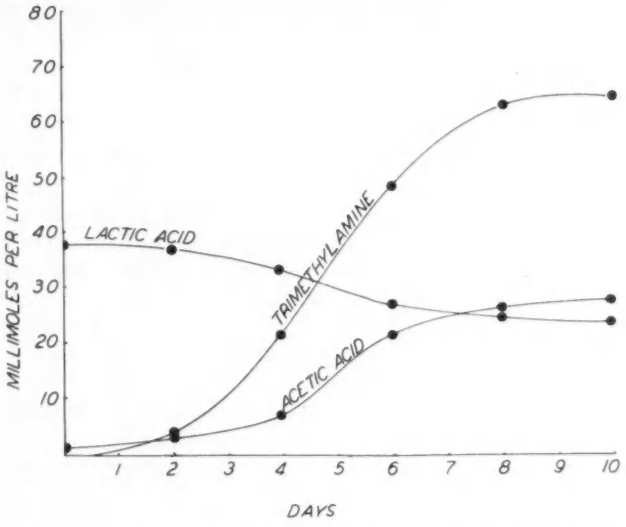

FIGURE 3. Changes in lactic acid, acetic acid, and trimethylamine in cod muscle press juice stored at 2°C. in the absence of air.

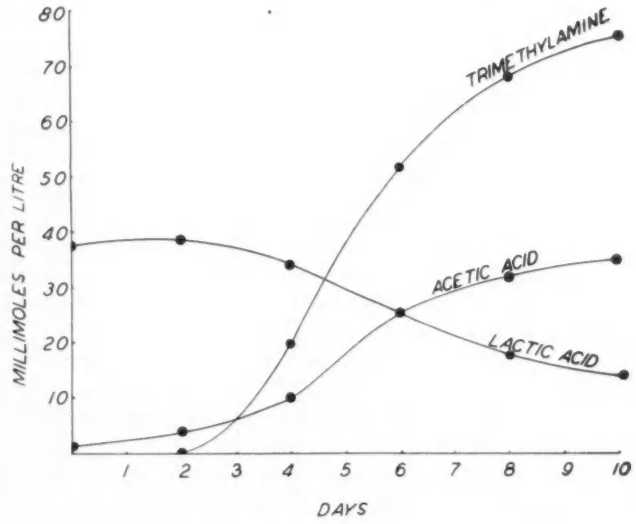

FIGURE 4. Changes in lactic acid, acetic acid, and trimethylamine in cod muscle press juice stored at 2°C. in the presence of air.

other tests. Like the trimethylamine test, it has the advantage of starting at a relatively low and constant level, and increasing with spoilage. In fish containing a comparatively large percentage of trimethylamine oxide, it has no advantage over the trimethylamine test, but if the trimethylamine oxide is low it might provide a more reliable guide to the state of preservation.

SUMMARY

Acetic acid has been found to be produced in spoiling cod muscle press juice. It has as its precursor lactic acid, which by oxidation with trimethylamine oxide in the absence of air yields acetic acid and carbon dioxide. In the presence of air less than the equivalent amount of carbon dioxide is produced. The fact that, in the absence of air, acetic acid and carbon dioxide are produced in equivalent amounts, leads to the conclusion that both are derived at least for the most part from lactic acid. Since their increase is more than equivalent to the decrease in lactic acid it is concluded that part of the latter is produced from some unknown precursor and that the reactions precursor→lactic acid→acetic acid go on concurrently. Neither glycogen nor reducing sugar has ever been demonstrated in sufficient quantity to lead to the belief that either of them is the unknown precursor.

REFERENCES

Beatty, S. A. *J. Fish. Res. Bd. Can.*, **4** (2) 63-68, 1938.
Beatty, S. A., and V. K. Collins. *J. Fish. Res. Bd. Can.*, **4** (5) 412-423, 1939.
Beatty, S. A., and N. E. Gibbons. *J. Biol. Bd. Can.*, **3** (1) 77-91, 1937.
Danielson, I. S., and A. B. Hastings. *J. Biol. Chem.*, **130**, 349-356, 1939.
Friedemann, T. E. *J. Biol. Chem.*, **123**, 161-184, 1938.
Friedemann, T. E., and J. B. Graeser. *J. Biol. Chem.*, **100**, 291-308, 1933.
Hillig, Fred. *J. Ass. Off. Agric. Chem.*, **22**, 414-418, 1939.
Hillig, Fred, and E. P. Clark. *J. Ass. Off. Agric. Chem.*, **21**, 688-695, 1938.
Malin, N. V. *Voprosy Pitaniya*, **7** (4-5) 148-155, 1939. (*C. A.*, **34**, 1762, 1940).
Watson, D. W. *J. Fish. Res. Bd. Can.*, **4** (4) 267-280, 1939.
West, E. S., F. H. Scharles and V. L. Peterson. *J. Biol. Chem.*, **82**, 137-153, 1929.

Studies of Fish Spoilage

IX. Changes in Buffering Capacity of Cod Muscle Press Juice

By V. K. Collins, C. C. Kuchel and S. A. Beatty
Atlantic Fisheries Experimental Station

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ABSTRACT

Cod muscle press juice during storage shows a decrease and later an increase in buffering capacity, both due to bacterial action. Reduction of trimethylamine oxide causes the decrease, and oxidation of lactic acid to acetic acid slightly lessens the effect. The increase is possibly entirely due to hydrolysis of protein by bacteria. Since the decrease occurs during the onset of spoilage, the buffering capacity gives useful data as to the state of preservation.

The obvious advantages of objective standards for the estimation of the quality or state of freshness of fish over organoleptic examination has led to an intensive search for methods of suitable sensitivity and of sufficient rapidity to be of practical value. Since one is concerned in such measurement not so much with the cause of spoilage as with the amount of damage done, chemical tests that measure, either directly or indirectly, changes in the muscle itself would appear to be on the soundest foundation. Of such chemical tests, designed to determine the state of preservation of sea fish, that of Stansby and Lemon (1933) is one of the most sensitive and rapid. These workers have shown that the buffering capacity of fish muscle between pH 6.0 and pH 4.3 decreases during storage, and they assign the decrease to changes in protein as a result of the growth of spoilage bacteria. Nickerson and Proctor (1935) reported that the buffering capacity of sterile muscle decreases with approximately the same rapidity as that of contaminated muscle. They stated that all their samples were frozen and defrosted before the period of storage and examination, thus introducing a factor that might explain their results. One would infer that this reduction in buffering capacity is not dependent on bacterial growth, but is an autolytic phenomenon, and therefore not of value as a measure of the destruction of, or undesirable changes in, the muscle, resulting from bacterial activity. Cutting (1938) published curves for decreases in buffering capacity which show a parallelism with trimethylamine oxide reduction and pointed out that trimethylamine oxide is a strong buffer for the range in hydrogen-ion involved, and that trimethylamine has hardly any buffering power. Since Beatty and Gibbons (1937) showed that the reduction of trimethylamine oxide is to all intents and purposes entirely the result of bacterial action, the whole question has become very confused and before either the test for freshness can be

used with confidence, or the reactions on which the test depend can be fitted into the general picture of fish spoilage, it is essential that one must determine (1) whether or not the change in buffering capacity is the result of bacterial action and (2) to what chemical reactions this change can be attributed.

METHODS

Preparation of Samples

The difficulty of obtaining sterile muscle in sufficient quantity for work has been pointed out in previous papers from this laboratory. Therefore, throughout the present work, expressate from cod muscle, that was ground and placed in the press previous to the onset of rigor, was used. As no precautions other than ordinary sanitation were taken to avoid contamination, the press juice always contained relatively large numbers of bacteria derived from fish slime and faeces as well as from land sources.

Cutting's results suggest very strongly that the lowering in buffering capacity is associated with trimethylamine oxide reduction, and it has been shown in this laboratory that this reduction is a form of bacterial anaerobic respiration. Therefore in the following experiments, unless otherwise indicated, spoilage was allowed to proceed in the absence of air. Controls were set up that were identical with the experimental lots, except that bacterial action was inhibited either by thorough impregnation with toluene, or by filtration through a Seitz E K filter.

Measurement of Buffering

During the earlier stages of the investigations the method of Stansby and Lemon (1933) was followed closely, hydrogen-ion measurements being made with the aid of a glass electrode. Later it was found that the elimination of protein and carbonates just previous to titration permitted the measurement of differences in buffering capacity too small to be detected by the original method. Expressates were treated with the protein precipitant of Steiner, Urban and West (1932), and filtered. Suitable aliquots of the filtrate were brought to pH 3.0 and shaken to remove carbon dioxide. They were then adjusted to pH 3.4 with standard barium hydroxide and the amount of this alkali required to bring the reaction to pH 6.0 was noted.

EXPERIMENTAL WORK

In the experiment, the results of which are shown in figure 1, lots of the same expressate were stored with and without toluene at the temperatures indicated, and the changes in buffering capacity were determined at intervals by means of the original Stansby-Lemon procedure. The samples containing no preservative all show a decrease in buffering capacity, followed by an increase. In so far as the authors are aware, the secondary rise has not been reported previously. It is of theoretical interest only, because by the time the buffering reached the minimum, the samples were very definitely spoiled as judged by sensory examina-

tion or by trimethylamine production. As is to be expected, this change is much more rapid at the higher temperature. The sample preserved in toluene at 2°C. shows no measurable change in buffering capacity. Thorough impregnation with toluene or sterilization by filtration has always entirely inhibited this change. Therefore one can conclude that with cod muscle expressate, and likewise almost certainly with the muscle itself, the Stansby-Lemon phenomenon is entirely the result of bacterial growth.

The influence of protein on the change in buffering capacity during storage is shown in figure 2. Here three lots of the same expressate were prepared, one as it came from the press, a second from which all proteins were removed by precipitate with alcohol and acetone, taking the filtrate to dryness to eliminate the precipitant and dissolving the dried residue in distilled water, and a third rendered almost

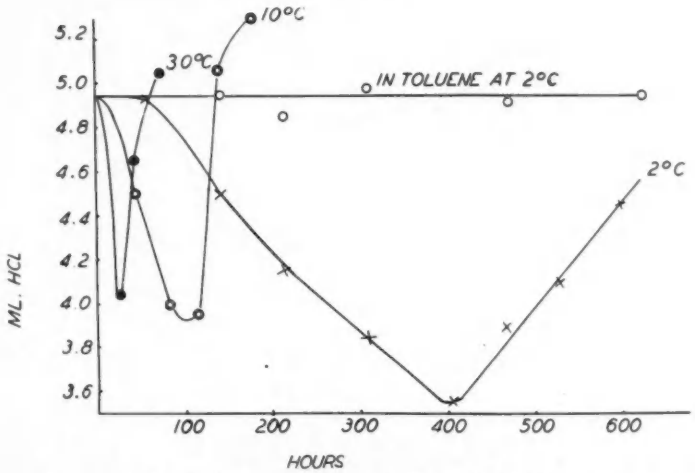

FIGURE 1. Changes during storage at various temperatures in the amount of 0.032 N hydrochloric acid required to bring one ml. of cod muscle press juice from pH 6.0 to pH 4.3.

protein free by dialysis through viscose casing. The dialysate was concentrated in vacuo, and the protein free dried filtrate was diluted with distilled water until each had approximately the same buffering capacity between pH 6.0 and 4.3 as the original expressate, and both were re-contaminated with a culture of organisms from cod slime and intestinal contents. The whole were incubated at 30°C., and measurements of the buffering capacity were made at intervals by the original method.

The curve for the untreated expressate is very similar to those of figure 1. Where no protein is present a decrease in buffering capacity occurs without any subsequent increase. Hence we can conclude that this decrease in buffering is due at least mainly to changes in non-protein constituents, while the increase is due to the presence of protein. The third curve, showing a decrease, then an increase, after which the buffering capacity falls off again, has a plausible explanation. In

this sample, protein was barely detectable. During the preliminary decrease, the bacteria are growing mainly on lactic acid (Beatty and Collins 1939). When the lactic acid reaches a low level or disappears, the proteins are attacked, the resultant peptones causing an increase in buffering. But since the amount of protein is small, the maximum is soon reached, after which as a result of the oxidation of the amino acids there is again a decrease.

If this explanation is true there should be an increase in ammonia about the time the buffering capacity increases. In figure 3 the rise in ammonia, as determined by the difference between the trimethylamine nitrogen and the total volatile basic nitrogen, is seen to occur approximately simultaneously with the increase in buffering capacity. Hence one can conclude with reasonable certainty that an

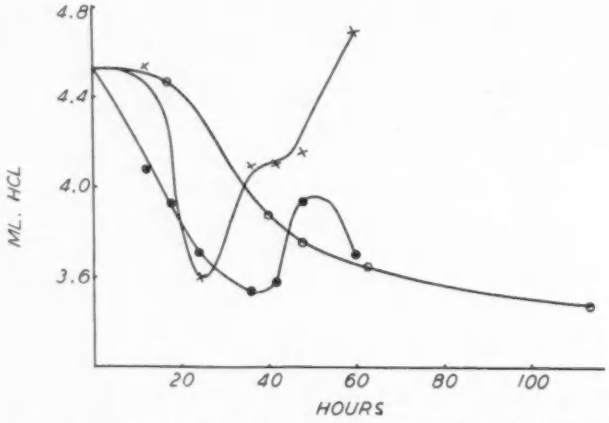

FIGURE 2. Changes during storage at 30°C. in the amount of 0.032 N hydrochloric acid required to bring one ml. of cod muscle press juice from pH 6.0 to pH 4.3. Unaltered press juice —— X —— X; protein-free press juice —— O —— O; press juice containing traces of protein —●—●.

important factor in this increase in buffer strength is the bacterial hydrolysis of the muscle proteins.

To what is the decrease in buffering capacity due? To test the relationship of this decrease with trimethylamine oxide reduction, determinations were made of trimethylamine by the Beatty-Gibbons (1937) modification of Lintzel's method and of buffering capacity by the original Stansby-Lemon technique in the same cod muscle expressate stored at 10°C. The results are shown in figure 3. The period of rapid trimethylamine production, and therefore of rapid trimethylamine oxide reduction, is seen to coincide with that of rapid decrease in buffering capacity. This confirms Cutting's observations.

The modification of the Stansby-Lemon technique previously referred to was devised to determine as accurately as possible the quantitative relationship between the reduction of the oxide and the change in buffering capacity. It has been used in the experiment, the results of which are shown in figure 4. Curves A and C

show the buffer changes in cod muscle expressate, sterilized by filtration, and unfiltered respectively, during storage at 2°C. The sterile sample shows a slight increase in buffering capacity undetected by the original procedure. The contaminated sample shows the usual fall and rise. Simultaneously with the above analyses, the trimethylamine oxide content of the sample was determined by the Beatty-Gibbons (1937) adaptation of Lintzel's method, and the buffering powers of the known trimethylamine oxide values were determined. These are shown in curves B and D. Since in the sterile sample trimethylamine oxide was not reduced, B is a straight line parallel with the base. The decrease in buffering capacity calculated from the reduction of the oxide, curve D, is more than the actual decrease, curve C.

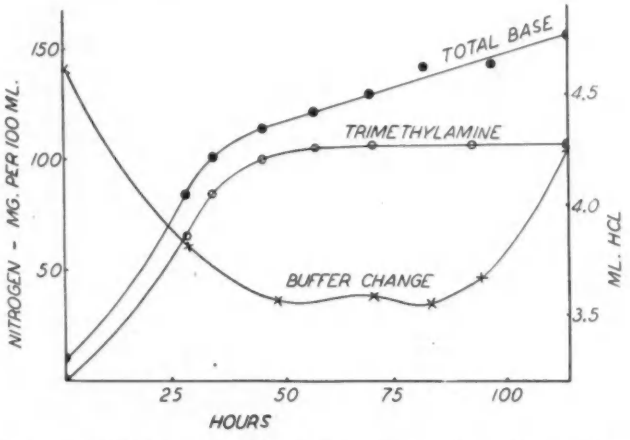

FIGURE 3. Changes in the amount of 0.032 N hydrochloric acid required to bring one ml. of cod muscle press juice from pH 6.0 to pH 4.3 in relation to changes in volatile bases during storage at 10°C.

In the preceding communication (Collins 1941) the relationship between lactic acid oxidation and trimethylamine production was shown. Since the ionization constant of lactic acid is 1.38×10^{-4}, and that of acetic 1.86×10^{-5}, the latter is more strongly buffered between pH 3.4 and pH 6.0 than is lactic acid. Furthermore acetic acid is produced in an amount more than equivalent to the decrease in lactic acid. Hence the oxidation of lactic acid to acetic acid tends to increase the buffering.

From known values of trimethylamine oxide, trimethylamine, lactic acid, and acetic acid, changes in buffering capacity were calculated and were compared with that as determined by the modified technique. The results are shown in figure 5.

The agreement between actual and calculated values in the anaerobic curves is particularly good, and while aerobic breakdown is more complicated, the two curves of buffer change in expressate stored in air show parallel trends, indicating

that the same oxidation-reduction reactions are responsible for the major part of the decrease in buffering capacity.

DISCUSSION

While the increase in buffering capacity during advanced spoilage has not been reported previously, it is only to be expected that the opening up of the protein molecule and the increase in carboxyl groups should produce such an effect. The finding is of value only in confirmation of previous work and as an addition to our knowledge of bacterial action on sea fish muscle. Since it is shown to take place after the fish has ceased to be an acceptable article of food, it is useless as a test of incipient spoilage.

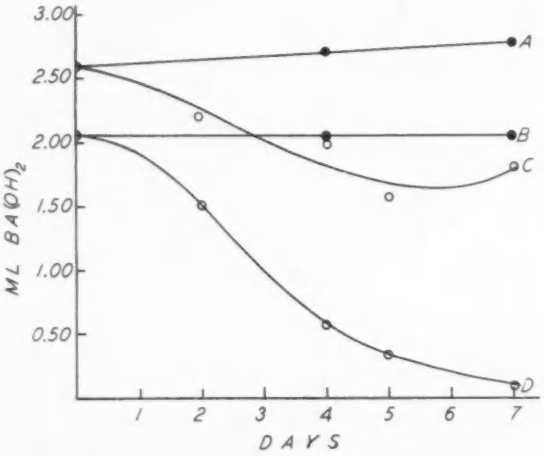

FIGURE 4. Changes in the amount of 0.040 N barium hydroxide required to bring one ml. of sterile and contaminated cod muscle press juice from pH 3.4 to pH 6.0 during storage at 2°C. A,—actual change in sterile press juice; B,—change in same press juice due to trimethylamine oxide reduction; C,—actual change in contaminated press juice; D,—change in same press juice due to trimethylamine oxide reduction.

The Stansby-Lemon observation, the decrease in buffering capacity (A value) has been shown to be associated with, and caused mainly by, changes in the concentration of trimethylamine oxide. Hence this test is an indirect measure of the rise in trimethylamine, and is dependent on the same reaction as the test for incipient spoilage developed in this laboratory. It would be of advantage, therefore, to discuss the relative merits of these in actual plant practice. We believe it will not be possible, at least for some time, to find a physical or chemical test sufficiently rapid to displace sensory examination, and that chemical tests will be used only in confirmation of this examination. To be on a sound basis, the final result of any test not only should give an accurate estimate of the damage done, but should provide as well a reasonably accurate forecast of "keeping" time. If it is

to be of practical use, the test should be sufficiently rapid to fit into a plant routine.

Of the tests for spoilage, those based on chemical changes resulting from bacterial growth are on the soundest bases. Three of the most useful of these are the Stansby-Lemon titre, the dimethylamine determination of Shewan (1938) and the trimethylamine determination of Beatty and Gibbons (1937). The Stansby-Lemon test and the measurement of trimethylamine are now shown to be two different measures of the same phenomenon. All three tests are on sound bases, because, in sea fish where these tests can be applied, the development of volatile bases renders the fish unpalatable, at least to a considerable portion of the con-

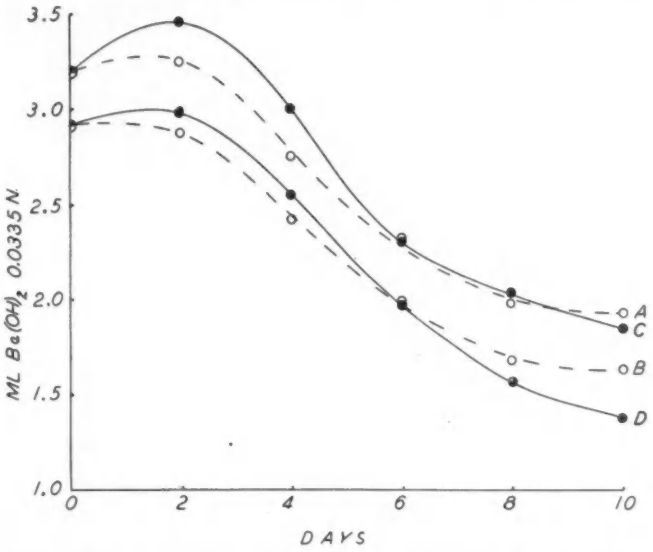

FIGURE 5. Changes in the amount of 0.0335 N barium hydroxide required to bring one ml. of cod muscle press juice from pH 3.4 to pH 6.0 in the presence and the absence of air during storage at 2°C. A,—muscle press juice stored out of contact with air; B,—theoretical values in same juice calculated from trimethylamine oxide, lactic acid and acetic acid concentrations; C,—muscle press juice in contact with air; D,—theoretical values in the same press juice calculated from trimethylamine oxide, lactic acid and acetic acid concentrations.

suming public, before other injurious changes become manifest (Beatty and Collins 1939). Since dimethylamine is produced before trimethylamine (Shewan 1938), and since the measurement of trimethylamine is more precise than the determination of a reduction in buffering capacity, the dimethylamine determination is the most sensitive and the Stansby-Lemon test the least. If reasonably accurate prognosis of the "keeping" time is required, both dimethylamine and trimethylamine should be determined. All three determinations are made with approximately equal rapidity, if dimethylamine is determined without distillation. Probably the determinaton of trimethylamine by the Beatty-Gibbons adaptation of the

Conway technique fits best into a laboratory routine, but because of the relatively long distillation period, the time that elapses between the taking of any one sample and the final result is longer than with either of the other methods.

SUMMARY

Cod muscle press juice during spoilage undergoes two changes in buffering between pH 6.0 and pH 3.4. Both these changes are the result of bacterial action. The first, a decrease in buffering capacity, is mainly the result of the reduction of trimethylamine oxide to trimethylamine, and the second, an increase, is due to protein hydrolysis. The latter change occurs after the fish is definitely spoiled and is useless as a measure of incipient spoilage.

The decrease in buffering capacity, being the result of bacterial growth, and being an indirect measure of the production of undesirable amines, gives useful information as to the state of preservation of the muscle, but, because the method is not as precise as the actual measurement of amines, and because the concentration in fresh fish muscle is not constant, the method is not as reliable as either the determination of dimethylamine or of trimethylamine.

REFERENCES

BEATTY, S. A., AND V. K. COLLINS. *J. Fish. Res. Bd. Can.*, **4**, 412-423, 1939.
BEATTY, S. A., AND N. E. GIBBONS. *J. Biol. Bd. Can.*, **3**, 77-91, 1937.
COLLINS, V. K. *J. Fish. Res. Bd. Can.*, **5** (3) 197-202, 1941.
CUTTING, C. L. *Gr. Brit. Dep. Sci. Ind. Res. Rep. Food Invest. Bd.*, **1937**, 78, 1938.
NICKERSON, J. T. R., AND B. E. PROCTOR. *J. Bact.*, **30**, 383-394, 1935.
SHEWAN, J. M. *Gr. Brit. Dep. Sci. Ind. Res., Rep. Food Invest. Bd.*, **1937**, 75, 1938.
STANSBY, M. E., AND J. M. LEMON. *Ind. Eng. Chem. (Anal. Ed.)*, **5**, 208-211, 1933.
STEINER, A., F. URBAN AND E. S. WEST. *J. Biol. Chem.*, **98**, 280-293, 1932.

The Fate of Trimethylamine Oxide and Trimethylamine in Man

By H. L. A. Tarr
Pacific Fisheries Experimental Station

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ABSTRACT

Normal human urine contains small amounts of trimethylamine oxide but no appreciable trimethylamine. Trimethylamine when administered orally appears mainly as trimethylamine oxide in the urine; trimethylamine oxide similarly administered is excreted unchanged, thus proving the existence in man of a system capable of oxidizing trimethylamine.

In the past the determination of the distribution of both trimethylamine and trimethylamine oxide in biological material has formed the basis for considerable research. It has been shown that, while trimethylamine oxide is almost invariably present in the muscle of sea fish, it is usually absent from fresh water fish (Hoppe-Seyler 1930, Kapeller-Adler and Krael 1930, Cook 1931, Beatty 1939). Recently, however, Lintzel, Pfeiffer and Zippel (1939) claim to have found from 9.1 to 92.3 mg. per cent of trimethylamine oxide in the tissues of certain fresh water fish (trout, tench and perch) and crabs. Kapeller-Adler and Vering (1932) found that, while trimethylamine is present in considerable, but variable, amounts in sea weeds and algae, it is absent from fresh water algae. Trimethylamine oxide was not found in any of these plants.

Information relating to the metabolism of trimethylamine and its oxide is somewhat meagre. The fate of trimethylamine and its oxide when fed to, or injected into, fish or mammals has been studied to a limited extent. Grollman (1929) found that trimethylamine oxide accounted for about one-half of the urinary nitrogen excreted by the goosefish (*Lophius piscatorius*). He suggested that the oxide might either have an exogenous origin, arising from the various Crustacea or fish upon which the goosefish feeds, or an endogenous origin, being formed from such compounds as betaine. Suwa (1909) injected trimethylamine oxide into rabbits and obtained both trimethylamine and trimethylamine oxide in the urine. Later Langley (1929) fed rabbits trimethylamine (as the hydrochloride) and found that 80 to 90 per cent was metabolized, probably being excreted as urea, the remainder being excreted unchanged. He did not state whether trimethylamine oxide was either tested for or detected in the urine.

As far as can be ascertained, the only published reports of experiments in which man was fed trimethylamine are those of Lintzel (1934), who stated that trimethylamine, when fed to man, appears chiefly as trimethylamine oxide in the urine. He found that normal human urine contains trimethylamine oxide,

the daily output being about 66 mg., but that the amount of trimethylamine excreted is negligible, and does not exceed 4 mg. per day. The present experiments were carried out in order to confirm and extend the work of Lintzel.

EXPERIMENTAL

Methods

Trimethylamine oxide ($(CH_3)_3N: O.2H_2O$; M.P. 96°C.) was made by oxidizing aqueous solutions of trimethylamine with hydrogen peroxide (Dunstan and Goulding 1899). Trimethylamine hydrochloride (Eastman Kodak) was recrystallized from methyl alcohol and ether prior to use (M.P. 270 to 272°C.).

Trimethylamine was determined using 10-ml. samples of urine and a technique previously described (Tarr 1940b). Trimethylamine oxide was determined by both biological and chemical reduction methods as follows.

Biological Reduction

Washed cells of culture 22 were employed as source of triamineoxidease enzyme (Tarr 1940b). Prior to use the urine samples were immersed for a few minutes in a boiling water bath in order to drive off any added toluene preservative present, and they were then cooled. Thunberg tubes received 5 ml. of urine; 3 ml. of bacterial cell suspension; 1 ml. of 0.2 M phosphate buffer pH 7.0 and 1 ml. of a solution containing equal parts of 0.1 M glucose and sodium lactate. The tubes thus prepared were evacuated, incubated for 24 hours at 25°C., and the trimethylamine determined on the whole contents as in previous work (Tarr 1940b). This method gave excellent results, and was especially useful in the case of samples of normal urine which contained verys mall amounts of trimethylamine oxide.

Chemical Reduction

Experiments carried out with a normal urine sample containing 0.01 M added trimethylamine oxide showed that prolonged reduction with hydrochloric acid and Devarda's alloy, as employed in the case of fish muscle juice (Beatty 1939), caused a progressive decrease in the amount of trimethylamine recovered. For the purpose of this work it has been found that a shorter reduction time than that employed by Beatty has given fairly satisfactory results. Two-millilitre samples of urine are reduced in 125 ml. flasks with 0.5 g. of Devarda's alloy and 2 ml. of 50 per cent hydrochloric acid for 10 minutes at 60°C. The reaction mixture is promptly cooled, diluted to 10 ml., and duplicate 3-ml. samples transferred to laboratory made Conway dishes. The trimethylamine present is then determined by distilling into 0.01 N acid after adding 0.5 ml. of formaldehyde and 1 ml. of 25 per cent sodium hydroxide solution. This method proved fairly satisfactory except in the case of samples of normal human urine in which the content of trimethylamine was very low. In the experiments to be described the urine of three individuals, all normal males and of ages 34(A), 34(B), and 37(C) years, has been examined. No fish was consumed for 24 hours prior to, or during, the collection of urine samples. The urine was stored over toluene at about 1.5°C. prior to examination.

Quantities Found in Urine

normally

The trimethylamine oxide content of four samples of urine from three individuals on a normal mixed diet is given in table I. It will be seen that trimethylamine oxide was present in definite, though variable, amounts in all these

Table I. Trimethylamine oxide content of normal human urine, and of human urine after oral administration of trimethylamine hydrochloride and trimethylamine oxide

Individual examined	Details regarding the urine sample	Trimethylamine oxide recovered (di-hydrate)			
		Biological reduction method		Chemical reduction method	
		Ml. 0.01 M trimethylamine per 5 ml. of urine	Mg. trimethylamine oxide in 24-hour urine sample	Ml. 0.01 M trimethylamine per 0.6 ml. of urine	Mg. trimethylamine oxide in 24-hour urine sample
A	1,190 ml. 24-hr. sample of normal urine	0.94 0.92	248 243 } 246	0.120 0.108	264 238 } 251
A	980 ml. 24-hr. sample of normal urine	0.76 0.78	166) 170) } 168		
B	Approx. 300 ml. of normal urine collected at random	0.90 0.98	240 262 } 251*		
C	Approx. 300 ml. of normal urine collected at random	0.40 0.43	107 114 } 111*		
A	1,375 ml. 24-hr. sample after oral administration of 1 g. of trimethylamine hydrochloride (exp. 1)	4.78 4.70	1460 †(1258) } 1448 1435 †(1235)	0.515 0.510	1308 †(1125) } 1302 1295 †(1115)
A	1,255 ml. 24-hr. sample after oral administration of 1 g. of trimethylamine hydrochloride (exp. 2)	4.34 4.18	†(1040) 1207 1165 } 1186 †(1002)		
A	1,270 ml. 24-hr. sample after oral administration of 1 g. of trimethylamine oxide (di-hydrate)	3.74 3.76	1060 1052 } 1056	0.420 0.428	988 1008 } 998

In the case of these individuals the values given are calculated on the supposition that the average daily urine excretion is 1,200 ml.

†The figures in brackets indicate the equivalent amount of trimethylamine hydrochloride.

urines, the daily output varying between about 110 and 250 mg., calculated as the di-hydrate. These values are higher than those reported by Lintzel (1934). No trimethylamine was found in duplicate 10-ml. samples of these urines by the technique employed.

AFTER ADMINISTERING TRIMETHYLAMINE

Experiment 1. 1 g. of trimethylamine hydrochloride was dissolved in 50 ml. of water. Individual A was fed approximately 5-ml. portions of this solution each hour over a 10-hour period, the urine being collected over a 24-hour period commencing with the first ingestion of the solution. The results, recorded in table I, show that the ingested trimethylamine is excreted practically quantitatively as the corresponding oxide. The recovery is considerably more than that expected from the amount of trimethylamine consumed, but the excess is undoubtedly due to the normal excretion of trimethylamine oxide. In this experiment no trimethylamine was detected in duplicate 10-ml. portions of the urine.

Experiment 2. Individual A was fed 10-ml. portions of a similar solution of trimethylamine hydrochloride to that used in experiment 1 every hour for 5 hours, the urine being collected as before. The results, given in table I, are similar to those obtained in the first experiment. However, in this case the trimethylamine hydrochloride was fed somewhat more rapidly, and the urine had a faint odour of trimethylamine. Analysis showed that in this case there was an average of 6.7 mg. of trimethylamine in the 24-hour urine sample.

AFTER ADMINISTERING TRIMETHYLAMINE OXIDE

1 g. of trimethylamine oxide dissolved in 50 ml. of water was fed to individual A as in the case of trimethylamine hydrochloride in experiment 1. The results, given in table I, show that the oxide is practically all excreted unchanged. No trimethylamine was detected in duplicate 10-ml. portions of the urine.

QUALITATIVE DEMONSTRATION

It has been shown in previous work that the biological method used in this work is specific for triamine oxides, other compounds containing closely similar chemical groups not yielding any volatile triamine base (Tarr 1940 a and b). Definite proof that the oxide present in the urine is the methyl compound was obtained in the following experiment.

1000 ml. of urine (experiment 1, trimethylamine hydrochloride fed) was placed in a 3 l. Erlenmeyer flask. After heating to 60°C., 50 g. of powdered Devarda's alloy was added, together with 100 ml. of 50 per cent hydrochloric acid. The flask was immersed in a water bath at 60°C. After 5 minutes 100 ml. of hydrochloric acid solution was added, the reduction process being permitted to continue for 15 minutes. The solution was promptly cooled, and the flask was then connected by means of a capillary tube to a suction flask containing 100 ml. of 0.1 N hydrochloric acid. 25 ml. of formaldehyde, 50 ml. of saturated NaOH solution and 50 g. of anhydrous sodium carbonate were added to the urine solution, and a stream of air washed in sulphuric acid was drawn rapidly through

the solutions for 24 hours. The solution in the receiving flask was then washed into a weighed crystallizing dish and evaporated over boiling water until a crystalline mass was obtained. After drying for several days over P_2O_5 and NaOH in a vacuum desiccator a yield of 724 mg. resulted, M.P. 269-270°C. A mixed M.P. of 269-270°C. was obtained on mixing with trimethylamine hydrochloride (M.P. 270-272°C.). From this result it is concluded that the compound in urine yielding the volatile base is trimethylamine oxide.

DISCUSSION

Though it is known that trimethylamine oxide occurs in considerable amounts in the muscle, blood and urine of most sea fish which have been examined by different investigators its role is as yet obscure. Ackermann, Poller and Linneweh (1926) showed that trimethylamine oxide is reduced to trimethylamine by both fresh and boiled mammalian liver. They assumed, as a result of certain observations, that this reduction was due to the thermostable sulphydryl system of Hopkins acting in conjunction with glutathione:

$$\underset{\substack{\text{(Glutathione reduced by the thermostable SH of tissues)}}}{2\,G-SH} + \underset{\substack{\text{(Trimethyl-amine oxide)}}}{(CH_3)_3N:O} \rightarrow \underset{\substack{\text{(Trimethyl-amine)}}}{(CH_3)_3N} + \underset{\substack{\text{(Oxidized glutathione)}}}{GS-SG} + H_2O$$

They also suggested that certain biological systems (dehydrogenases) capable of reducing trimethylamine oxide might exist. Recent work (Tarr 1939; 1940 a and b) has shown that certain bacteria are capable of reducing trimethylamine oxide to the corresponding amine according to the following equation:

$$\underset{\substack{\text{(Activated by triamine-oxidease enzyme)}}}{(CH_3)_3N:O} + \underset{\substack{\text{(Hydrogen from substrate activated by dehydrogenase enzyme)}}}{H_2} \rightarrow (CH_3)_3N + H_2O$$

On the other hand little is known regarding the biological oxidation of the free amine. Kapeller-Adler and Vering (1932) fed frogs and goldfish trimethylamine hydrochloride and found that in both cases the muscle contained trimethylamine in significant amounts. They were unable to detect trimethylamine oxide in the muscle. In fact, though trimethylamine oxide has frequently been demonstrated in sea fish there is as yet no published work which indicates that it arises from trimethylamine. The present work has demonstrated without question that there exists in the human body some system, or systems, capable of oxidizing trimethylamine to the corresponding oxide, and that both these compounds are relatively stable in this environment. The origin of the trimethylamine oxide occurring in normal human urine is not known, but it may arise from the breakdown of such compounds as betaine, ergothioneine, etc., which may be conceived to yield trimethylamine which is subsequently oxidized. It will be interesting to ascertain whether systems capable of reducing trimethylamine oxide (hydroxytrimethyl ammonium hydroxide, Hattori 1940) and of oxidizing the reduced form, exist in fish tissues. Since the amine occurs in sea

fish largely or entirely as the oxide (Beatty 1938) it would appear that if such a reversible system exists, the component which effects oxidation is much more powerful than that which occasions reduction. So far attempts by the writer to reduce trimethylamine oxide with fresh fish muscle anaerobically have failed. It is hoped that further investigation may throw some light on the function of trimethylamine oxide in various forms of marine life.

SUMMARY

Both biological and chemical reduction methods have shown that the urine of man on a normal mixed (fish-free) diet contains about 110 to 250 mg. of trimethylamine oxide (di-hydrate) in a 24-hour sample.

Trimethylamine hydrochloride when administered orally to man appears largely as the corresponding oxide in the urine, but small amounts may appear as the free amine when fed rapidly.

Trimethylamine oxide when similarly administered appears unchanged in the urine.

The existence of a system in man capable of oxidizing trimethylamine to its oxide is thus proven.

The possible role of trimethylamine oxide as a reversible oxidation-reduction system is discussed.

REFERENCES

ACKERMANN, D., K. POLLER AND W. LINNEWEH. *Ber. Deut. Chem. ges. B*, 2750-2751, 1926.
BEATTY, S. A. *J. Fish. Res. Bd. Can.*, **4**, 63-68, 1938.
J. Fish. Res. Bd. Can., **4**, 229-232, 1939.
COOK, A. S. *Canad. Chem. Metall.*, **15**, 22, 1931.
DUNSTAN, W. R., AND E. GOULDING. *J. Chem. Soc.*, **75**, 1004-1011, 1899.
GROLLMAN, A. *J. Biol. Chem.*, **81**, 267-278, 1929.
HATTORI, Y. *J. Pharm. Soc. Japan*, **60**, 24-45, 1940 (*Chem. Abst.* **34**, 3387, 1940).
HOPPE-SEYLER, F. A. *Z. Biol.*, **90**, 433-466, 1930.
KAPELLER-ADLER, R., AND J. KRAEL. *Biochem. Z.*, **221**, 437-460, 1930.
KAPELLER-ADLER, R., AND F. VERING. *Biochem. Z.*, **243**, 292-309, 1932.
LANGLEY, D. *J. Biol. Chem.*, **84**, 561-570, 1929.
LINTZEL, W. *Klin. Wochenschr.* **13**, 304, 1934. (*Biochem. Z.*, **273**, 243-261, 1934).
LINTZEL, W., H. PFEIFFER AND I. ZIPPEL. *Biochem. Z.*, **301**, 29-36, 1939.
SUWA, A. *Arch. ges. Physiol.*, **129**, 231-239, 1909.
TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1939.
J. Soc. Chem. Ind., **59**, 349, 1940a.
J. Fish. Res. Bd. Can., **5** (2) 187-196, 1940b.

Tryptic Enzymes from Certain Commercial Fishes

By W. W. Johnston
Atlantic Fisheries Experimental Station

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ABSTRACT

An examination of fish digestive organs showed that only the pyloric caeca and intestine showed a sufficiently high yield and a sufficiently high activity to make their commercial use feasible. The intestine had approximately one-quarter the activity of the caeca. Mackerel caeca showed the highest activity and yield of any organ in four species of fish examined. No activating effect parallel to that of mammalian enterokinase on trypsinogen was demonstrated. By comparing the hydrolysis products of casein by pyloric caeca, intestinal mucosa, and hog pancreas, evidence was adduced showing that the enzymic constituents of these preparations are similar.

The work on the utilization of enzymes from dehydrated portions of the fish digestive tract was undertaken in the expectation that dried preparations of certain digestive organs might be of use commercially as leather bates. In a previous communication (Johnston 1937) it was shown that dried pyloric caeca of codfish furnish an enzyme preparation similar to that of hog pancreas and suitable for leather bating. The present work attempts to determine the total proteolytic activity of the digestive organs of certain commercial fish, mainly cod, haddock and hake. It deals also with the effect of mixing enzymic material from various organs, and with a comparison of the type of breakdown effected by the enzymes of fish pyloric caeca, fish intestinal mucosa, and hog pancreas.

METHODS

Preparation of Enzymes

The crude dry preparation of the pyloric caeca enzyme was made as described by Johnston (1937) except that the ground tissue was treated with 3.5 instead of 1.3 times its weight of acetone and ether.

The enzymes from the stomach were prepared as follows. After removal from the fish, the stomach was slit open, and if not empty, the food contents were removed. It was then rinsed by dipping it once in a beaker of water. The linings were separated from the heavy muscle walls and immersed in 3.5 times their weight of a solution, acetone 90 per cent, ether 10 per cent. They were then treated in the same manner as the pyloric caeca preparations.

Enzymes from the intestine were prepared by squeezing the intestines free of faeces, slitting open, and rinsing by dipping once in a beaker of water. These

were then dehydrated by immersion in acetone-ether followed by spontaneous drying at room temperature.

A crude enzymic preparation from the liver was prepared by adding to the livers 3.5 times their weight of acetone and one half their weight of ether. After standing for one half to one hour, the livers were squeezed as dry as possible and immersed again in one half of their weight of ether. After squeezing, the residue was dried spontaneously at room temperature.

Enzymic preparations from the gall bladder and pancreas were prepared in a manner similar to that used for the pyloric caeca.

The hog pancreases were dehydrated by immersion in 3.5 times their weight of a solution, acetone 90 per cent, ether 10 per cent, followed by spontaneous drying at room temperature.

Measurement of Activity

The substrate for determination of enzyme activity was composed of a 5 per cent casein solution held at pH 8.0 with a borate buffer and at a temperature of 40°C. To 50 c.c. of this substrate was added 10 c.c. of a 5 per cent enzyme "solution" and the activity of the preparation was determined by following the progress of the hydrolysis of casein. The progress curve was obtained by taking samples of the digest at increasing intervals, precipitating the undigested casein with trichloracetic acid and determining the residual non-protein nitrogen in the filtrate. The method is given in greater detail by Johnston (1937).

Amino nitrogen determinations were carried out on trichloracetic acid filtrates after elimination of any free ammonia present. This was done by treating aliquots of the filtrate with a slight excess of magnesium carbonate, and boiling for five minutes. The boiled liquor was then slightly acidified with acetic acid, made to volume, and the amino nitrogen determined manometrically in the apparatus of Van Slyke (1929), using the reagents and modifications described by Kendrick and Hanke (1937).

Ammonia determinations were carried out on aliquots of the trichloracetic filtrates in the Parnas apparatus, as modified and described by Beatty and Gibbons (1936).

EXPERIMENTAL

Enzyme Concentration and Casein Decomposition

The activity of enzymes is often determined by drawing tangents to the progress curve for the reaction at zero time. The slope of the tangent is taken as a measure of the rate of reaction. The difficulty with the method lies in the fact that two observers may draw slightly different tangents. Hence in the following pages, the activity of various enzyme preparations was compared by a calculation of the amount of nitrogen rendered soluble by one gram of the preparation acting for 20 minutes under the defined experimental conditions. However, before this procedure was permissible it had to be shown that the relationship between quantity of enzyme added to the substrate and the amount of substrate decomposition as thus measured, was linear under the experimental conditions employed throughout this work.

Figure 1, which shows the number of milligrams of casein nitrogen made soluble by increasing amounts of the same enzyme preparation in 20 minutes, demonstrates that such a relationship does exist when working under the experimental conditions prescribed.

Enzymes from Various Organs

Stomach Preparations

The stomach is regarded sometimes as a reservoir in which to hold food. It is known of course that considerable digestion occurs in this organ. In the mammals and in some species of fish (Vonk 1929; Yonge 1931; Babkin et al. 1935), this digestion is carried on at an acid reaction at which tryptic enzymes are inactive.

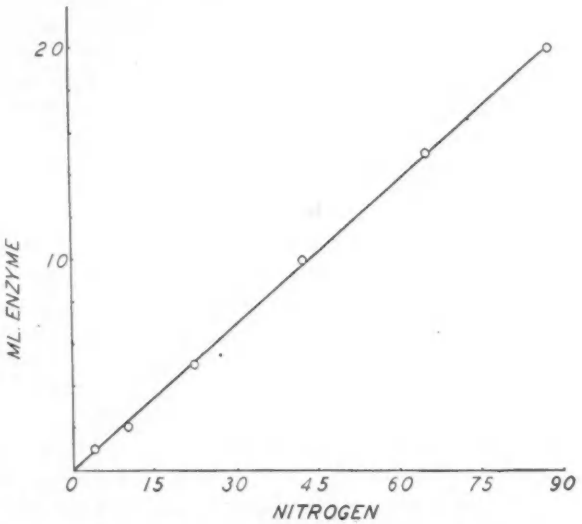

Figure 1. Relationship between enzyme concentration and digestion.

In the course of this investigation, however, an attempt was made to determine if the fish stomach has any tryptic activity. This was done by employing the dried stomach mucosa powder in the same way that the dried pyloric caeca preparations were used. Under these conditions one gram of the preparation from cod, haddock and hake increased the soluble nitrogen from casein in 20 minutes by 8.6, 5.6 and 7.6 mg. respectively. The yields of dried powder as a percentage of the weight of the fish were variable but the averages were 0.19, 0.22 and 0.26 per cent respectively. These low yields in conjunction with the relatively low activity of the preparations prohibit the use of the stomachs of these fish as sources of commercial bating material.

As further work, which will be reported in a subsequent communication, has shown that very active pepsin and rennin preparations can be made from

cod and haddock stomachs, it appears likely that the slight tryptic activity observed resulted from a contamination of the stomach by the tryptic enzymes of the pyloric caeca.

LIVER PREPARATION

Yields of dry preparation by the acetone-ether method of defatting and dehydration were relatively high from fish livers but the tryptic activity of the resulting preparations from the three species of fish dealt with was insignificant.

GALL BLADDER PREPARATIONS

Yields of a dry powder on dehydration of the gall bladder were very low, being of the order of 0.007 per cent. The activities of these preparations were very variable. Cod showed no activity while one gram of the haddock preparation increased the casein nitrogen under the defined conditions by 24.2 mg., and a hake preparation under the same conditions increased it by 5.7 mg. Since the low yields do not make the use of the gall bladder feasible as a commercial source of enzymic material, these values have not been confirmed. The fact that the values are so different might indicate that the activity of the bile may have arisen by contamination from some other source.

PANCREATIC PREPARATIONS

The functions of the pancreas in certain fish seem to have been assumed at least partially by the pyloric caeca, and the pancreas remains as a vestigial organ. Accordingly, the yields that can be obtained from this organ in cod, haddock or hake are insignificant, being of the order of magnitude of 0.002 per cent of the weight of the gutted fish. The activity of one dried preparation only from codfish was tested. It showed that one gram of the dried preparation would render 23.4 mg. of casein soluble in 20 minutes under the described experimental conditions. The activity of dried pancreas preparations from hake or haddock was not tested but the very low yields which could be obtained in conjunction with its relatively low activity would render this organ totally unsuitable as a raw material for the preparation of a commercial bate.

PYLORIC CAECA PREPARATION

The yield of dry pyloric caeca powder which can be obtained from cod or haddock amounts to about 0.3 per cent of the weight of the gutted fish, from hake about 0.2 and from mackerel 0.37 per cent.

A number of factors which affect the activity of the pyloric caeca enzymes were dealt with previously (Johnston 1937). When working with preparations which are influenced by several uncontrollable factors, as a consequence of which their activities vary over wide ranges, it is difficult to strike a satisfactory average. Nevertheless, bearing this limitation in mind, the following average activities will be found useful as a guide to the order of magnitude one might expect from caeca preparations of the following fish. One gram of the dried caeca powder increased the casein soluble nitrogen in 20 minutes by the following amounts: cod 150 mg.; haddock 72 mg.; hake 163 mg.; mackerel 459 mg.

Extreme values for different lots of codfish treated in the same manner were 75 mg. and 252 mg. The individuals in each lot of haddock examined were small, the round gutted fish averaging only 600 grams. The extreme values found were 47 mg. and 180 mg. Only two lots of mackerel were examined in striking the average shown but each lot was composed of a representative sample from approximately 50 kilograms of ungutted fish. The increases in casein-soluble nitrogen brought about by one gram in 20 minutes from the two different lots were 378 mg. and 540 mg.

INTESTINAL PREPARATIONS

The amounts of a dried enzyme preparation which were made from the intestines were as follows: cod 0.16 per cent, haddock 0.17 per cent and hake 0.12 per cent. The activity of these preparations was determined on casein substrates under the defined experimental conditions. The results showed that the cod preparation increased the non-precipitable casein nitrogen on an average by 79 mg. in 20 minutes, the haddock by 29 mg. and the hake by 54 mg. For convenience in reference the data concerning yields and activity of the various organs of these fish have been summarized in table I. The yields shown represent the number of pounds of a dry preparation which can be produced from 100 pounds of gutted fish.

TABLE I. Yields on the basis of gutted weight and enzyme activity of organs of various fishes

SPECIES	STOMACH		PYLORIC CAECA		INTESTINAL MUCOSA		LIVER		GALL BLADDER		PANCREAS	
	YIELD	ACTI-VITY	YIELD	ACTI-VITY	YIELD	ACTI-VITY	YIELD APP.	ACTI-VITY	YIELD	ACTI-VITY	YIELD	ACTI-VITY
Cod	0.19	8.6	0.30	150	0.16	79	6.0	0.0	0.007	0.0	0.002	23
Haddock	0.22	5.6	0.30	72	0.17	29	6.0	0.0	0.005	24.2		
Hake	0.26	7.8	0.20	163	0.12	54	6.0	0.0	0.007	5.7		
Mackerel			0.37	459								

While the values shown represent the averages of many individuals of each species, it must be pointed out that in extreme cases activity values for one individual are sometimes more than three times as high as for another, and hence the figures shown must be accepted as being only of the correct order of magnitude. An examination of the table shows, however, that in so far as the preparation of leather bates is concerned the only organs having commercial possibilities are the pyloric caeca and the intestines. From the latter one might prepare about one quarter as much enzymic activity as from the pyloric caeca. From an economic point of view, however, the increased labour in cleaning these may not make their employment feasible.

Chesley (1934) examined a number of fish and found that the pancreas

of most of those which he studied showed at least as much protease activity per gram of tissue as the caeca. In view of his finding, the low activity of the cod pancreas found in these experiments is somewhat striking.

Synergism Between Enzymes

In the mammals it is a well known fact that trypsin as excreted has only a low activity, and that this may be greatly increased by an excretion known as enterokinase. Similarly T. Ôya and S. Yokota (1933) have shown that the pancreatic trypsin-like protease of catfish is strongly activated by a kinase from the intestinal mucosa. In view of these facts it seemed possible that some activating effect might be found between preparations of the different organs of the cod or haddock. Accordingly extracts from the pyloric caeca were mixed with extracts from the stomach and intestinal linings. These mixed extracts were allowed to remain at room temperature for one hour, then added to a casein substrate, and their activities determined. In no case was any synergistic action observed. Since, however, the enzyme preparations with which we were dealing were not prepared from strictly fresh organs, it was felt that perhaps the enzymes had become fully activated before dehydration. To test this hypothesis a number of cod were brought alive to the laboratory and, after slaughter, their organs were immediately removed and immersed in acetone. Five per cent "solutions" of these organs dried at room temperature were prepared, equal volumes mixed together, and after standing for one hour at room temperature, were allowed to react on casein substrates. A summary of the results is shown in table II.

Table II. Protease activity of various organs of cod and mixtures of the same

Organ	Found	Calculated
Pyloric caeca	115	—
Intestinal mucosa	77	—
Stomach mucosa	7.3	—
Liver	0.0	—
Gall bladder	0.0	—
Pyloric caeca+intestinal mucosa	94	96
Pyloric caeca+stomach mucosa	58	61
Pyloric caeca+gall bladder	61	58
Liver+gall bladder	0.0	0.0

In table II by "activity" is meant the number of milligrams of casein nitrogen rendered soluble in 20 minutes by one gram of the different preparations acting at 40°C. From the table it is apparent that in no case was any activating effect produced by one organ on another. Similar experiments were also carried out on extracts of the dried organs of haddock and hake, but in no case was any activation observed by the action of one organ on another.

COMPARISON WITH HOG PANCREAS, PYLORIC CAECA AND INTESTINAL MUCOSA

According to older views trypsin was regarded as an individual protease system which decomposes proteins into polypeptides and some amino acids. More recent work has shown however that, far from being an individual substance, trypsin is a complex system. Waldschmidt-Leitz and Purr (1929) separated it into four fractions by absorption under different conditions on aluminum and iron hydroxides. Two of these fractions were made up of a dipeptidase and an amino polypeptidase, having the same properties as gut erepsin. The remainder was further fractionated into a proteinase and another peptidase. The latter peptidase requires a free carboxyl group for its action. Waldschmidt-Leitz and Purr regarded the protinease as the classical trypsinogen. More recently however, Waldschmidt-Leitz and Akabori (1934) demonstrated the existence of two proteinases in pancreatic tissue. This has also been confirmed by Kunitz and Northrop (1935, 1936) who crystallized both chymotrypsinogen and trypsinogen from active pancreatic extracts. It appears therefore that trypsin is a complex enzymic system composed of at least two proteinases and two or more peptidases. Hence it becomes interesting, not only from a scientific point of view, but also from a consideration of the use of tryptic enzymes as bating materials, to compare the decomposition products of the usual bating enzymes with these from fish, and thus to gain some information regarding the relative amounts of the various components in the trypsin preparation. In case of a trypsin containing a large proportion of proteinases, one would expect the increase in amino acid nitrogen to be relatively small when compared with another sample of trypsin containing a relatively large proportion of peptidases. Conversely a trypsin containing a relatively low concentration of proteinases and a high concentration of peptidases would show a low activity as measured by the "soluble casein nitrogen technique" while showing a relatively high proportion of amino acid nitrogen. Similarly an enzyme preparation containing significant quantities of amidases would be expected to liberate considerable ammonia from a digest. Among animal organs, amidases are usually found in the liver, but recently a specific amidase has been prepared from the gut (Waldschmidt-Leitz and Balls 1930). It was considered necessary therefore to follow the liberation of ammonia (as well as the increase in amino nitrogen and total nitrogen soluble in trichloracetic acid) by the intestinal enzymes of the cod, and to compare these with similar values obtained as the result of the activity of hog pancreas and cod pyloric caeca. Figures 2, 3 and 4 show the increases in ammonia, amino nitrogen and total trichloracetic acid soluble nitrogen after increasing periods in the presence of hog pancreas, pyloric caeca and intestinal mucosa respectively.

In no case was the amount of ammonia liberated by the enzymes greater than one per cent of the total nitrogen made soluble. It is clear therefore that the amount of amidases is very low if not absent, and in any case is comparable and insignificant in all preparations. The data plotted graphically in figures 2, 3 and 4 are shown in different form in table III. The first and second columns show for example that when hog pancreas has digested a casein substrate to

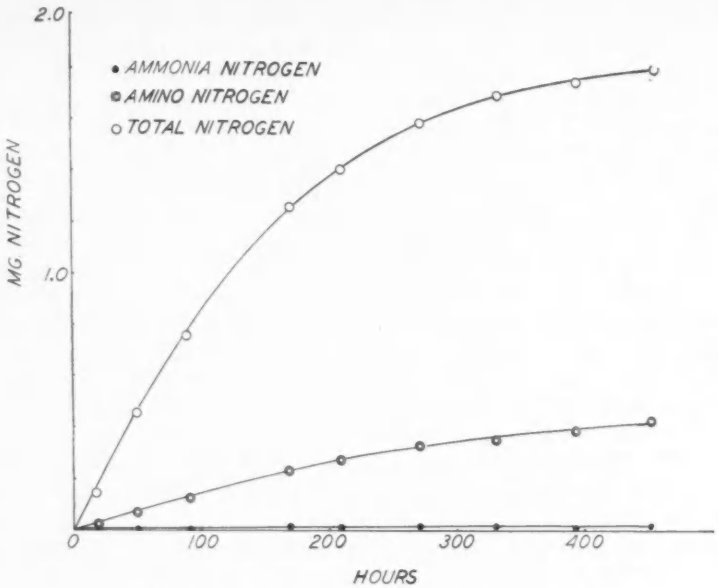

FIGURE 2. Digestion of casein by hog pancreas.

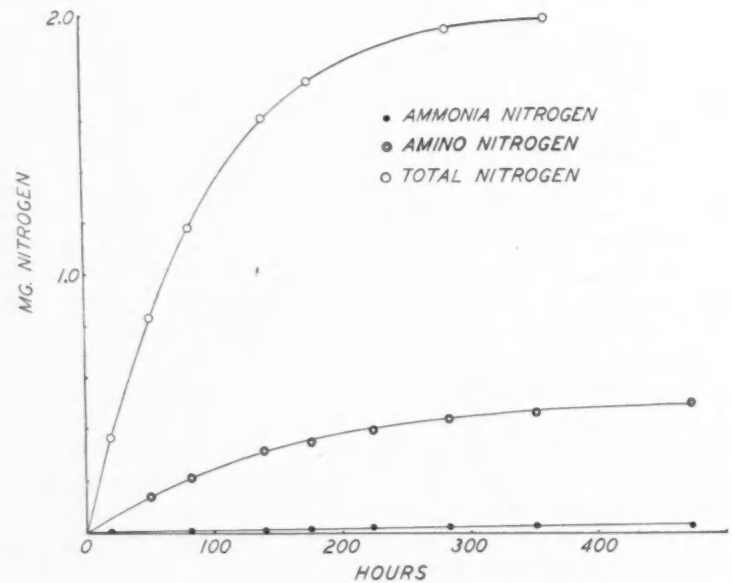

FIGURE 3. Digestion of casein by pyloric caeca.

such an extent that 20 per cent of the nitrogen in the digest will no longer precipitate with trichloracetic acid, 13.7 per cent is in the form of amino nitrogen. Parallel values are shown when the non-precipitable nitrogen has reached levels of 30, 40, 50, 60 and 70 per cent. Similar values are shown in columns 3 and 4 for cod pyloric caeca and cod intestinal mucosa preparations.

As may be observed from examination of table III, the proportion of

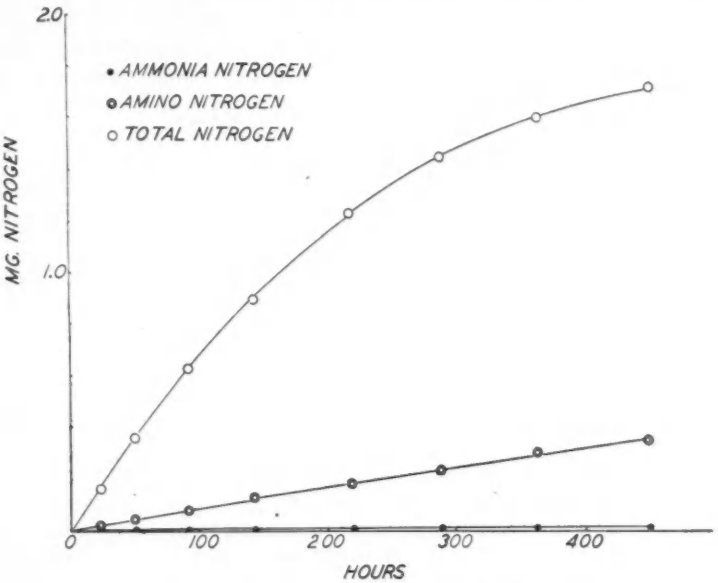

FIGURE 4. Digestion of casein by cod intestinal mucosa.

TABLE III. Amino acid nitrogen as percentage of total nitrogen made soluble in trichloracetic acid by different enzymes

Soluble nitrogen as percentage of total nitrogen in digest	Amino acid nitrogen as percentage of total soluble nitrogen		
	Hog pancreas	Cod pyloric caeca	Cod intestinal mucosa
20	13.7	13.9	13.2
30	16.0	16.1	13.2
40	17.1	17.6	14.0
50	18.5	18.6	15.4
60	19.8	19.7	17.0
70	22.0	21.2	19.1

amino acid nitrogen to total soluble nitrogen continues to increase slowly for the duration of the experiment, but never increases to much more than twenty-five per cent of the total nitrogen made soluble in trichloracetic acid. As the pyloric caeca and hog pancreas liberate almost the same proportion of amino acid nitrogen, it must be assumed that the relative proportion of proteases to polypeptidases is approximately the same in both enzymic preparations. Intestinal preparations on the other hand liberate a slightly lower proportion of amino groups. All however liberate proportions of amino nitrogen of the same order of magnitude and hence must be comparable in composition. Therefore there seems to be no reason why either the pyloric caeca or intestinal mucosa enzymes could not replace hog pancreas as a commercial leather bate.

SUMMARY

An examination of various organs of certain commercial fish has shown that only the pyloric caeca and the intestinal mucosa show any possibilities for the preparation of commercial leather bates.

About one quarter as much tryptic activity can be prepared from the intestine as from the pyloric caeca.

In some instances low variable tryptic activity was observed in bile and stomach preparations.

Of the various organs of the different species of fish examined the pyloric caeca of the mackerel was the most active. Its yield per pound of fish was also somewhat higher than that of cod, haddock or hake.

No activation of the pyloric caeca enzymes could be produced by extracts from the other organs of the fish.

A comparison of the hydrolysis products of cod caeca, cod intestine and hog pancreas preparations shows that the proportion of ammonia nitrogen liberated in each case represents about one per cent of the total. It also shows that the proportion of amino nitrogen to total nitrogen runs almost exactly parallel with pyloric caeca and hog pancreas and nearly so with intestinal enzymes. This constitutes evidence that the proportion of enzymic constituents in the different crude preparations is similar.

REFERENCES

BEATTY, S. A., AND N. E. GIBBONS. *J. Biol. Bd. Can.*, **3**, 77-91, 1936.
BABKIN, B. P., A. F. CHAISSON AND M. H. F. FRIEDMANN. *J. Biol. Bd. Can.*, **1**, 251-259, 1935.
CHESLEY, L. C. *Biol. Bull.*, **66**, 133-144, 1934.
JOHNSTON, W. W. *J. Biol. Bd. Can.*, **3**, 473-485, 1937.
KENDRICK, A. B., AND M. E. HANKE. *J. Biol. Chem.*, **117**, 161-174, 1937.
KUNITZ, M., AND J. H. NORTHROP. *J. Gen. Physiol.*, **18**, 433-458, 1935.
J. Gen. Physiol., **19**, 991-1007, 1936.
ŌYA, T., AND S. YOKOTA. *J. Imp. Fish. Inst. Japan*, **28**, 97-103, 1933.
VAN SLYKE, D. D. *J. Biol. Chem.*, **83**, 425-427, 1929.
VONK, H. J. *Z. vergl. Physiol.*, **9**, 685-702, 1929.
WALDSCHMIDT-LEITZ, E., AND S. AKABORI. *Z. physiol. Chem.*, **228**, 224-234, 1934.
WALDSCHMIDT-LEITZ, E., AND A. K. BALLS. *Ber.* **63**, 1203-1211, 1930.
WALDSCHMIDT-LEITZ, E., AND A. PURR. *Ber.* **62**, 2217-2226, 1929.
YONGE, C. M. *J. Conseil*, **6**, 175-212, 1931.

Cyclical Abundance and Birds versus Salmon

By A. G. Huntsman
Fisheries Research Board of Canada

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ABSTRACT

American mergansers and belted kingfishers that eat large parr, particularly during the low water of dry summers, fulfilled the prediction (based upon statistics) that the periodic scarcity of Atlantic salmon is due to a factor operating on the salmon when near the smolt stage. One year's bird control more than doubled the number of descending smolts and correspondingly increased the sea catch of salmon related to the experimental river, as demonstrated by the occurrence of fish marked as smolts in the river. Bird control may thus remedy periodical scarcity. This serves to explain the favourable influence on numbers of fish that man's presence has been observed to have.

CYCLICAL ABUNDANCE OR PERIODICAL SCARCITY

At a time when rapid expansion of markets was causing fear that overfishing was depleting or even exhausting stocks of Atlantic salmon, there was a pronounced fluctuation in their abundance from New Brunswick to Labrador. In the seventies of the nineteenth century their numbers were reported to be greater than had ever been known, and then in 1880 and 1881 smaller than for any years that could be remembered. Critical examination of records showed "that by far the largest decrease in salmon is in those salted in barrels, which are all taken in the eastern counties, in Cape Breton and on the Labrador coast, where the rivers are neither obstructed by mill dams or afflicted with sawdust or poachers to any extent." (Rogers 1881, p. 144). This not only absolved man from being to blame for the catastrophic decline, but even suggests that man's effect may have been good, since the decline was greatest where man's operations were least.

This puzzle soon passed out of recollection, but the collection of statistics of the fishery, which had been started just after Confederation, in 1868, continued to be made annually. Renewal of the marked fluctuations in the twenties of this century again focussed much attention on the matter and resulted in the usual complaints of excessive fishing. Griswold (1929) sought an explanation for the very poor angling season of 1928 and found a nine-year cycle in abundance in both angling and net catches of New Brunswick and Quebec, which Phelps and Belding (1931) corroborated by a careful study of the Restigouche angling records. Huntsman (1931) independently found in the statistics of the net catches a periodic scarcity of 9.6 years on the average over a sixty-year period, in close correspondence with the period for abundance in the fur-bearing animals of the Canadian North West, as shown by the figures published by Hewitt

(1921). Knowledge of the length of life in both river and sea of the salmon in different localities in combination with the statistical evidence as to the exact year of local scarcity formed the basis for the prediction (Huntsman 1931) that the periodical scarcity was due to some factor acting unfavourably upon the salmon of a particular year-class about the time of their descent to the sea as smolts, and at the same time acting favourably on the younger fish of subsequent year-classes present in the water with those unfavourably affected. A periodicity of fairly definite length might well have an astronomical cause, but neither sun-spot cycles nor tidal periods evoked to explain the phenomenon corresponded with the length of the salmon cycle.

BIRDS AND WATER HEIGHT AFFECT ABUNDANCE

The problem was then attacked in a different fashion,—by studying the factors affecting the numbers of the young salmon, and it was found (White 1936) that fish-eating birds were important; in particular, the belted kingfisher and the American merganser proved to be rearing their young on the salmon parr of such a salmon stream as the North East Margaree river of Cape Breton island. Since they took mainly the older fish, in their last year of parr life, they were seen (Huntsman 1937) as likely to constitute the factor operating unfavourably "near the time of the descent of the salmon from the river as smolts", and as also to be likely to act favourably on the younger fish in the water by removing the older fish, the competitors and perhaps the enemies of the former. It was considered that the birds did not have a pronounced effect in a particular year by virtue of their being very abundant in that year, but rather through the water of the stream being low and clear in that year and thus permitting them to remove the fish easily. White (1938, p. 52) then showed that this is true, that when the water is high the kingfisher takes trout rather than salmon, the former being more in the upper and shallower parts of the river system. To corroborate this view of the importance of depth of water, some correspondence was found (Huntsman 1937) between water height of the North East Margaree river during the summer months and quantities of salmon taken in the Margaree region three years later, when the affected fish had reached the size for capture.

BIRD CONTROL GIVES MORE SMOLT

As water height might readily affect the numbers of the young salmon in other ways than through the birds, and as there are many other possible factors acting at different stages in the life history to determine the abundance of the adult salmon, it seemed somewhat doubtful whether control of the birds could be demonstrated as affecting the catches of salmon; but it did seem that it would be possible to recognize the effect on the numbers of the young, by trapping and counting the smolts as they descended the stream. Accordingly, Forest Glen brook, a tributary of the North East Margaree river, was selected for an experiment. As it was found that kingfishers and mergansers reared their young on it in the early summer of 1936 and toward the end of the summer took them elsewhere, presumably because they could no longer get sufficient

food, it seemed evident that the birds had had nearly their full effect; so a count was made the next spring (1937) of the remaining large parr that were changing into smolts (White 1939) and 1,834 were found descending. The fish-eating birds of the district were kept down as thoroughly as possible for a full year and another count was made in 1938, which gave 4,065 smolts, an increase of 120 per cent, indicating a decidedly favourable effect for bird control.

It can, however, be properly affirmed that, with so many factors operating, the experiment should have been a much longer one, making counts for a number of years with the birds given full scope, and then for a number of years with the birds kept down, so as to permit a comparison of averages for the two conditions. However, it had been shown (Huntsman 1931) that, for successive periods of as much as ten years, the average quantities of the adults taken yearly may vary greatly, which would affect the usefulness of averages unless these were for much longer periods. It seemed better, therefore, to rely upon the above experiment that gave the greatest possible contrast in the two conditions (many birds vs. no birds) and upon an analysis of the attendant circumstances.

Fortune had favoured us. It might be argued that *more spawners* were responsible for there being more smolts in 1938 than in 1937 although no particular relation had been found (Huntsman 1931, p. 39) between the quantity of salmon in one year and that of their progeny in the proper subsequent year. To test this idea we must first know when the smolts for each of these years were spawned.

Approximately 92 per cent of the Forest Glen smolts for each year are three-year-olds, and 7 per cent two-year-olds (White 1939, p. 22), which means that the 1937 smolts were spawned chiefly in 1933 but also in 1934, and that the 1938 smolts similarly were spawned in 1934 and 1935. There seemed to be an equal abundance of the newly hatched young in the significant summers of 1934, 1935 and 1936 following the spawnings of 1933, 1934 and 1935, but no accurate counts were or could be made. The young were, however, considered in each year to be more than the river could bring through to the smolt stage. Also, since the net fishery takes only about 25 per cent of the salmon along the Margaree coast (Huntsman 1939) and the bulk of the fish enter the river too late to be very desirable for food or even for sport, and are at that time usually well protected from poaching by high water, there is always likely to be an abundance of spawning fish. However, to relieve all doubt as to any effect of spawning on the results of the experiment, it happens that the spawning stock of Margaree salmon was dropping rather than rising from year to year in the significant years as judged by the catch of the Margaree region:—1933, 1,458 cwt.; 1934, 1,077 cwt.; 1935, 856 cwt. (Fish. Stat. Canada 1934, 1935, 1936). This means that there should have been more spawners instead of less for Forest Glen brook in 1933 and 1934 to produce three-year-old and two-year-old smolts in 1937 than there were in 1934 and 1935 to produce such in 1938.

Since *water height* seems definitely of significance for quantity of fish, and may act in varied ways, it could have made interpretation difficult if it had favoured the 1938 smolts. It happened, however, that the water height in the

summers of 1935, 1936 and 1937, the significant years, was progressively lower instead of higher, the mean discharge of the North East Margaree river at Frizzleton for June, July and August in 1935, 1936 and 1937 being 412, 268 and 228 sec.-ft. respectively (private communications from K. G. Chisholm, District Hydraulic Engineer).

An incidental matter was the count. Winter conditions as well as cost made it desirable to use a temporary fence and trap of somewhat light construction, which could scarcely withstand the sudden, severe floods to which the stream is subject. An exceptional flood did occur and permit smolts to descend that could not be counted, but this was in 1938 only, so that the increase of smolts in that year over the previous one may have been greater but could not have been less. It is believed not to have been much greater, since the trap was out of commission for only a day or so as the run was developing.

BIRD CONTROL GIVES MORE ADULT SALMON

While we have every reason to rely upon the soundness of Mr. White's experiment in demonstrating the value of bird control, it is of definite importance to ascertain, if possible, whether any effect can be observed of the reduction in birds on the numbers of the adult salmon. The smolts of 1937 were to be expected to return to the river, possibly a few as grilse in 1938, chiefly as two-sea-year salmon in 1939 and partly as three-sea-year salmon in 1940, and the smolts of 1938 similarly in 1939, 1940 and 1941. In so far as they returned to Forest Glen brook, arrangements could have been made for trapping and counting them in those years. It was considered that the results to be expected would not justify the expenditure of so much time and labour as would be involved. The smolts were marked both in 1937 and in 1938 by the removal of the adipose fin with a razor blade and by scratching a cross in the skin of the left side of the body. It was thought that these fish might be recognized when taken by the fishermen and thus permit a comparison of the two years. Somewhat more than 27,000 smolts were also marked in the North East Margaree river in 1938, but only by removing the adipose fin. In all 544, that is 2 per cent, two-sea-year salmon with the adipose fin missing and with no other mark were reported taken by nets in the sea during the season of 1940. On this basis the Forest Glen smolts of 1937 should have given 37 recaptures in 1939, and those of 1938, 80 recaptures in 1940. Although the salmon of the Margaree region were specially examined in both 1939 and 1940 by those receiving them for storage and shipment, only one fish corresponding to the Forest Glen marking was found in 1939 and none was found in 1940. It might be thought that regeneration of the skin would result in the cross on the side being overlooked, though Mr. White has had no difficulty in recognizing such marking in returning grilse at Moser river. This would result in the Forest Glen smolts of 1938 being confused with those marked in the North East Margaree river in that year, but it would not explain the failure to find more than one two-sea-year fish with missing adipose fin in 1939. That 10 three-sea-year fish so marked were found in 1940 indicates that two-sea-year fish with missing adipose fin were probably overlooked in 1939.

Owing to the movements of the birds from one part of the river to another, their control for the experiment on Forest Glen brook was generally effective for the whole of the North East Margaree river. Also, certain of the fishery guardians assisted considerably in reducing the numbers of the birds along that river. Since the latter is the chief salmon river of the region, it seemed possible that the effect of the control might be recognized in the fishermen's catches in 1940, the most significant year, in spite of the North East Margaree salmon being mixed in the sea with salmon from other rivers. Study of the sea behaviour of the Margaree salmon (Huntsman 1931, 1936 and 1939) has given some background for judging of this effect.

There was firstly the question as to whether, if periodical scarcity was caused by low water permitting easy removal of the older parr by birds, control of the birds would remove or mitigate the bad effects of low water on the salmon population. These effects had been recognized in the fishery statistics. Would the latter show their removal or mitigation? Fortunately for the purpose, 1937 was the second and the drier of two very dry summers, so that a very poor salmon fishing season was to have been expected on the Margaree coast in 1940. The catch for north Inverness county from 1934 to 1940 in comparison with the mean summer discharge of the North East Margaree river from 1931 to 1937 is shown in figure 1. It will be seen that the catch was alternately high and low up to 1939 in correspondence with alternate high and low summer discharge up to 1936, and that there was also a general downward trend. Then, although the discharge was even lower in 1937 than in 1936, the catch in 1940 rose abruptly to a value close to the two highest for the period. It may be concluded that bird control can obviate the bad effects of low water and may thus be a remedy for periodical scarcity of salmon that caused so much concern in 1880-1881, 1919-1920 and 1928 particularly.

There was secondly the question as to whether it could not be shown that the improvement in catch ascribed to bird control was in the fish from the Margaree where the control had been carried out rather than in those from other rivers. The catch for the section of the coast related to the Margaree river is also given in the figure, although only from 1936 to 1940. It shows a decidedly more marked rise in 1940 than does that for the larger area.

The condition can be brought out in another way. The smolts marked in the North East Margaree river in 1938 gave by their recapture in 1940 a rather clear picture of where the Margaree salmon are taken in the sea, in that year at least. While these marked fish were taken from Pleasant bay, 35 miles to the northeast, to the coast of Antigonish county, 75 miles to the southwest, they were in high proportion in the catch only from Whale head, 2½ miles to the southwest, to Cheticamp point 12 miles to the northeast (1 mile = 1.61 km.). We have satisfactory detailed records of the catches for the Cape Breton part of this distribution, but only for the years 1936, 1937 and 1940. In table I these catches are shown by districts and for each district the percentage that the catch for 1940 makes of the average for 1936 and 1937 is given together with the percentage of the marked Margaree salmon in the 1940 catch. On the whole the 1940 catch is high where the proportion of Margaree salmon is large.

The Margaree river discharges between AuCoin point and Whale head, and it will be seen that from this Margaree river district both height of the 1940 catch and proportion of Margaree salmon drop off abruptly to the southwest, and but slowly to the northeast. This is in correspondence with the movement of

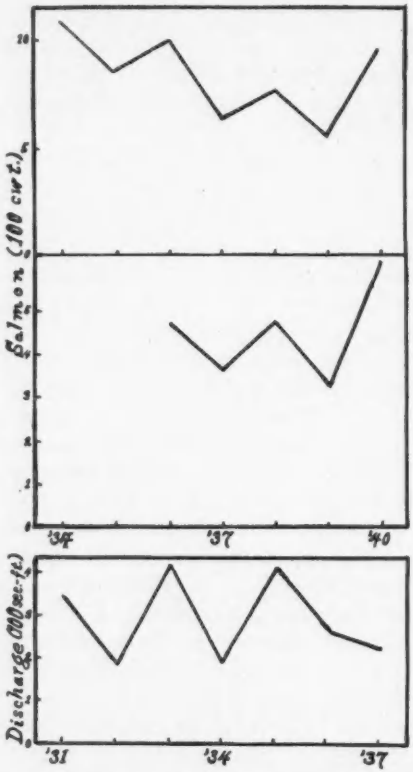

FIGURE 1. Mean summer (June to August) discharge of the North East Margaree river related to weight of salmon landed on the coast three years later, both for north Inverness county (upper graph) and for the part (Whale head to Cheticamp point) with most Margaree salmon (middle graph). The effect of bird control in 1937 appears as a high landing of salmon in 1940. (Discharge data from Dominion Hydrometric Bureau, north Inverness salmon catches from the Department of Fisheries, and salmon catches for coast related to the Margaree river from the records of those receiving and shipping the salmon).

the water from the Margaree river northeastward along the coast. In figure 2 the data for the catches of the various districts are plotted according to distance from the Margaree river. It will be evident that the heavy catches are from the Margaree river northeastward in correspondence with the predominant importance of the Margaree river and the direction of movement of its water, and that only for such districts does the 1940 catch rise above the average

TABLE I. Salmon in districts on Inverness coast (west coast of Cape Breton island). Relation of 1940 catches to the averages for 1936 and 1937 and proportions of marked Margaree salmon in the 1940 catches

	Mi.	Km.	1936 Catch (cwt.)	1937 Catch (cwt.)	1940		
					Catch (cwt.)	Relation to 1936-37 catch (%)	Marked Margaree salmon (%)
Cape St. Lawrence to Pollett cove	10	16	0	0	0	0	0
Pollett cove to White capes	16	26	91	96	77	82	2.7
White capes to Cheticamp is.	12	19	131	114	141	115	2.7
Cheticamp is. to Friar hd.	8	13	169	125	206	141	7.2
Friar hd. to Au Coin pt.	4½	7	188	141	253	154	9.1
Au Coin pt. to Whale hd.	3½	5½	110	94	153	150	11.0
Whale hd. to Broad cove	14	22½	156	98	101	80	4.0
Broad cove to Low point	43	69	130	93	80	71	1.8
Low point to Ship harbour	10	16	0	0	0	0	0

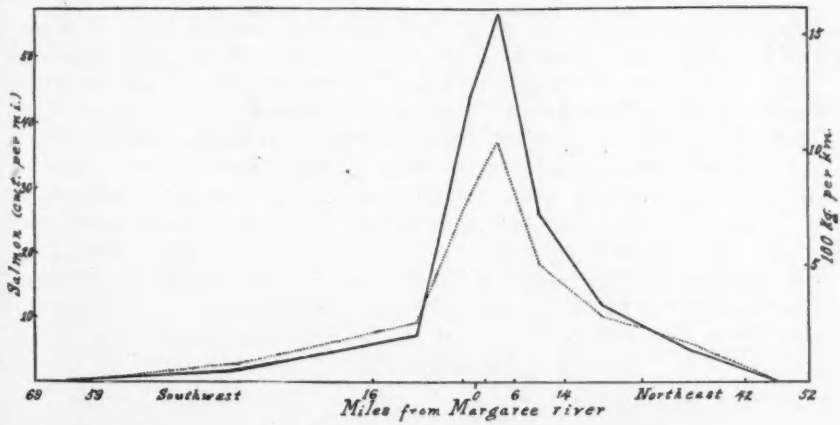

FIGURE 2. Hundredweights of salmon per mile in 1940 (——) compared with average for 1936 and 1937 (····) in various districts on the western coast of Cape Breton island (Inverness county), plotted on the basis of their distances southwest and northeast of the point of discharge of the Margaree river.

for 1936 and 1937. Such information concerning fishing intensity as we have is to the effect that outside the districts from Cheticamp point to Whale head the fishery was not prosecuted during 1940 as well as usual, so that the abundance of the fish in the outer districts was doubtless relatively greater than the catches in that year would indicate. Poor catches discourage fishing and thus the catches tend to over-emphasize scarcity of fish. The conclusion reached is that the relatively high catch for north Inverness in 1940 is properly

ascribed to bird control since the catch is highest in the districts with most Margaree salmon and since the control was exercised on the Margaree river.

The amount of the increase due to bird control is not capable of accurate determination, but the catch in 1940 should without bird control have been even lower than in 1939. Since it was almost double the 1939 catch for the coast related to the Margaree river (fig. 1), such an increase is probable. For the river as a whole the increase should not be as great as for Forest Glen brook, where the smolts increased 120 per cent, since the control was more complete on that brook.

There will also have been an effect on the angling catch in the river. This is not measurable, since that catch varies mainly with the way in which the fish enter and ascend the river, and this is dependent upon the very variable rainfall during the season. It was probably doubled by the bird control.

PROBLEM NOT SETTLED

Although the effectiveness of bird control in increasing the numbers of Margaree salmon has been well established, the general problem of producing a large number of salmon is far from being settled. Bird control, itself, can merely be said to offer good possibilities for increasing salmon production. Obviously it will be of particular value only where the birds are preying heavily on the young salmon. But there is also the point that we as yet know the results of only a single year of control. Continued control may give a different result. On the one hand it would be expected that for fish that live for three years in the streams as parr, there would be advantage in protecting them from birds for more than their final year. On the other hand elimination of the birds may result in the increase in numbers of other enemies or of competitors for food. On Forest Glen brook there was evidence that in one year's control, the trout had increased as well as the salmon and some of them were large enough to be eating salmon smolts, as Mr. White discovered. There is evidence also that, if very numerous, the large parr will eat the smallest salmon and that one year-class may keep down the numbers of the succeeding year classes that are in the stream at the time. The effects of bird control should be carefully followed.

EFFECT OF MAN

Man's direct effect on the large salmon through poaching, erection of dams and pollution is fully appreciated and may be overemphasized. That he may have a beneficial effect seems never to have been considered, but is definitely suggested by Rogers' statement quoted above that the scarcity of salmon in 1880 was most extreme where man's influence was least. If periodical scarcity is the result, as has been concluded, of thorough removal of the young salmon by birds when the water is low and clear, will man prevent or reduce such removal? Mr. White has repeatedly drawn our attention to the abundance of young salmon and trout in the vicinity of dwellings along the Margaree during dry seasons. This was interpreted as the result of the birds being driven away from or avoiding man's vicinity, which seems to be true. There is also the

probability that there is more food for the young salmon in streams from which the shading trees have been removed by man.

Forest Glen brook runs through a valley which once supported quite a number of families. No one lives there now and but little evidence of man's occupation now remains. Similar changes have occurred and are occurring in other parts of the Margaree river system. It would appear that this has lessened and will lessen the production of young salmon. Bird control and tree removal will reverse the process.

SUMMARY

Periodical scarcity of Atlantic salmon, more pronounced in districts where man's influence was least, was predicted from analysis of statistics of the catches as caused by some factor acting unfavourably on the young salmon about the time of their descent to the sea as smolts.

Kingfishers and mergansers take the salmon parr during their last year in the river and to that degree qualify as the factor for periodic scarcity.

The low, clear water of dry summers permits thorough removal of the young salmon by birds, and, so far as tested, dry summers have preceded the periodical scarcities at the proper interval.

Experimental elimination of the birds from a stream more than doubled the number of salmon smolts descending subsequently. Analysis of the attendant circumstances eliminates extent of spawning and height of water as causative factors for the increase.

In the proper year subsequent to the bird control the catch of salmon in the sea was much greater than was to be expected from the dryness of the season of control. Detailed analysis of the catch in relation to the occurrence of salmon, which had been marked as smolts in the experimental river, shows that the catch was proportionately highest where were most fish from the river along which the birds had been reduced.

It is concluded that control of birds may remedy periodical scarcity.

Man's presence is seen as favouring young salmon by driving or frightening away the fish-eating birds.

REFERENCES

FISHERIES STATISTICS OF CANADA **1933**, **1934**, and **1935**, *Dom. Bur. Stat.*, 1934, 1935 and 1936.

GRISWOLD, F. G. *Salmo salar*. 1-28. Norwood, Mass. (privately printed), 1929.

HEWITT, C. G. The conservation of wild life in Canada. xx+344, New York, 1921.

HUNTSMAN, A. G. *Bull. Biol. Bd. Can.*, **21**, 1-99, 1931.
Bull. Biol. Bd. Can., **51**, 1-20, 1936.
Trans. Roy. Soc. Can., Ser. 3, **31**, 17-27, 1937.
Bull. Fish. Res. Bd. Can., **57**, 1-75, 1939.

PHELPS, E. B., and D. L. BELDING. A statistical study of the records of salmon fishing on the Restigouche river. 1-39, New York, 1931.

ROGERS, W. H. **11***th Ann. Rep. Min. Mar. Fish.*, **1880**, App. 9, 142-162, 1881.

WHITE, H. C. *J. Biol. Bd. Can.*, **2**, 299-309, 1936.
J. Fish. Res. Bd. Can., **4**, 48-52, 1938.
Bull. Fish. Res. Bd. Can., **58**, 1-30, 1939.

Temperatures and Salinities under the Ice in a Shallow Inlet

By A. W. H. Needler
P.E.I. Biological Station

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ABSTRACT

In Bideford "river", an arm of Malpeque bay, soon after ice forms a thin layer of water of very low salinity develops with a minimum temperature above the freezing point of the salter water below. Convectional mixing of surface and bottom waters is largely prevented. The temperature of the latter seldom approaches its freezing point closely and rises during the winter to approach that of the water in contact with the ice.

So little has been published on hydrographic conditions under ice that it seems worth while to present even incomplete information. Data were obtained incidental to oyster investigations at the Prince Edward Island Biological Station which suffice for a preliminary picture only.

The hydrography under the ice is important in connection with the distribution and survival of animals and plants. It will be seen that the presence of ice influences both temperature and salinity and makes possible the presence of water above its freezing point throughout the winter. A special significance is given to this by Sparck's (1927) observations that winter mortality among oysters was especially marked where waters were free from ice during the frost period. He attributed this to super-cooling and the formation of ice-crystals in the oysters.

GENERAL CONDITIONS IN BIDEFORD RIVER

Bideford river is a branch of Malpeque bay which is itself a sheltered inlet with an area of about 16,000 hectares (40,000 acres) and a maximum depth of about 15 metres (about 50 feet). For a map and for further information about Malpeque bay reference may be made to an earlier paper (Needler 1931).

There is relatively little fresh water entering the inlet. Salinities over 25 per mille occur at the extreme head in spite of the tidal range of only 0.6 to 1.1 m. (2 to 3½ ft.). Temperatures reach an extreme of about 25°C. in the summer and ice covers the inlet for three and one-half to about six months in the winter.

COLLECTION OF DATA

The data were obtained principally at a station about a mile from the head of tide, where the depth was about 4 m. (about 12 ft.). Salinity samples were

taken with a glass bottle, lowered closed and opened at the given depth. The cap was of the spring type of an ordinary citrate bottle. Salinities were determined with Knudsen's hydrometers. Temperatures of the water sufficiently near the surface were taken with an ordinary surface thermometer graduated in tenths of a degree and at greater depths with a Negretti & Zambra reversing thermometer.

TABLE I. Water temperatures before and after ice formation

Date	Temperature (°C.)		Freezing point (°C.) of water sample		Ice
	Surface	Bottom	Surface	Bottom	
1930					
Nov. 19	3.3	3.3	−1.5	−1.5	Not present
Nov. 28	3.9	2.8	−1.5	−1.5	Present
Dec. 3	−0.7	−1.1	−1.5	−1.5	Present
Dec. 17	−0.5	−1.3	−0.9	−1.6	Present
1931					
Nov. 9	5.3	5.3	−1.5	−1.5	Thin patches
Nov. 14	5.6	5.6	−1.5	−1.5	Thin patches
Nov. 19	5.7	6.1	−1.5	−1.6	Present, thin
Nov. 24	7.7	7.2	−1.5	−1.5	Not present
Nov. 28	2.7	4.3	−1.5	−1.6	Not present
Dec. 5	−0.3	−0.4	−1.5	−1.5	Present
1932					
Nov. 22	3.7	3.9	−1.6	−1.6	Not present
Dec. 2	0.7	0.5			Not present
Dec. 9	0.6	1.1			Not present
Dec. 23	−0.8	−1.2	−1.5	−1.7	Present
1933					
Oct. 24	8.0	7.7	−1.6	−1.6	Not present
Nov. 6	2.0	2.6	−1.5	−1.6	Present
Nov. 21	0.0	−0.7	−0.3	−1.6	Present
1934					
Nov. 14	1.5	4.4	−1.3	−1.5	Not present
Dec. 9	0.3	0.4	−0.7	−1.6	Present
Dec. 30	0.2	−0.7	−0.2	−1.6	Present

Unfortunately, cutting a hole in the ice unavoidably disturbs the surface water layers. The considerable hollow that is made is filled suddenly with water which rushes in when a hole is finally broken through the bottom layers of ice. Furthermore, the lowering of sample bottles and reversing thermometers disturbs the water to some extent so that temperatures and salinities at various depths cannot be obtained with great accuracy in this way. The salinity will not always correspond with the temperature. This inaccuracy is most serious in the surface

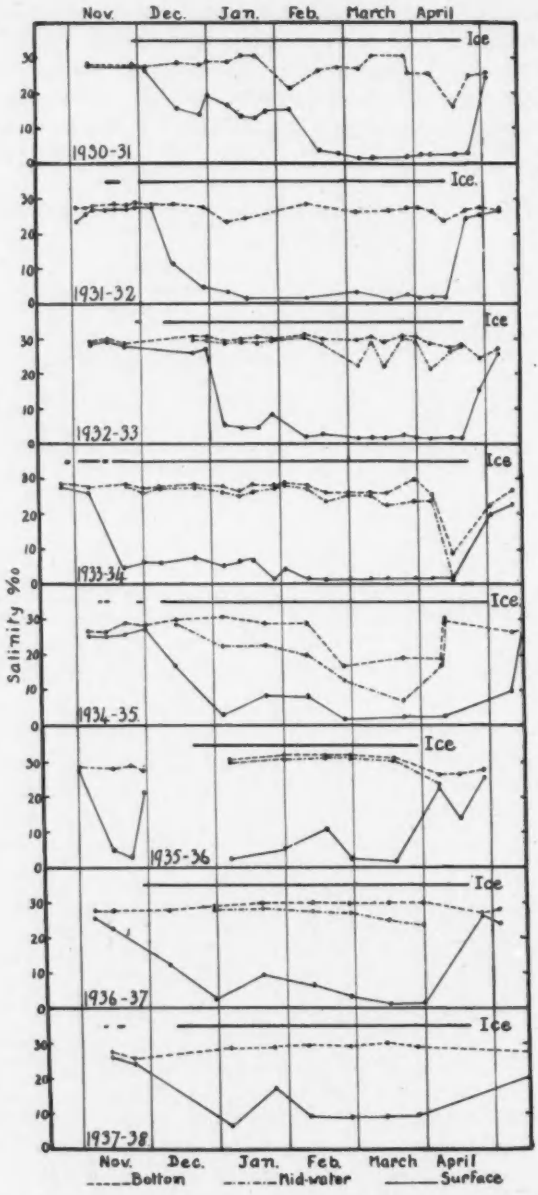

FIGURE 1. Salinities at surface, mid-water and bottom (about four m.) at the principal observation station about one mile below the head of tide in Bideford river, Malpeque bay. No salinities below 1.8 per mille were determined and salinities shown at this value may be lower.

layers where, as will be seen below, there is a sharp stratification. Refinement would be desirable, using some methods which would not disturb the water so much, but the inaccuracy of the present data is not sufficient to invalidate the general conclusions.

FORMATION OF ICE

Ice first forms on the inlet usually during cold calm weather. It forms first at the head of the inlet and later towards the open. In the years 1930 to 1937 the first ice was formed at the station under observation as early as October 12 and as late as December 19. Frequently ice will be formed and disappear, either through melting or wave action, two or three times before it comes to stay.

Ice is frequently formed while the temperature of most of the water is well above the freezing point as shown by the data in table I. It is not suggested that the water in actual contact with the ice is above its freezing point in these cases, but the layer of water in actual equilibrium with the ice is too thin to be recognized in our observations. Apparently heat can be lost by the ice to the atmosphere sufficiently quickly to maintain a thin layer of ice in spite of the proximity of warm water.

STRATIFICATION IN SALINITY UNDER THE ICE

During open water there is little or no stratification except immediately after heavy rains. This occurred in November, 1935 (figure 1), the rains being from November 12 to 14 and 17 to 19. As shown in figure 1, a surface layer of low salinity always develops soon after the formation of ice and persists until the ice leaves. The salinity of the bottom water usually remains high until the spring, when a decrease may occur before the disappearance of the ice and accompanied by an increased inflow of fresh water.

The thickness of the surface layer of water of low salinity is indicated by more detailed data shown in figure 2. In the six cases presented there the thickness of the fresher layer varies from less than 15 cm. (6 in.) to about 1 m. (3 ft.). In each case there is a sudden transition to more saline water. Numerous observations at mid-depth (figure 1) have shown that conditions there are usually closely similar to those at the bottom, thus confirming the thinness of the fresher surface layer.

Judging from the observed effectiveness of wave action in producing mixing in open water, its prevention by ice is an important factor in the development of the surface layer of fresher water. The source of the latter is apparently mainly inflow from brooks or seepage since the freshest surface water at the middle of Malpeque bay was repeatedly found to be much saltier (March 16, 1933, 22.9‰; April 1, 1933, 27.6‰; February 19, 1934, 30.3‰; March 17, 1934, 17.5‰) than was being found in Bideford river. Fresher surface water will be produced also by thawing of the under surface of the ice, and by melting of its upper surface to make water which, augmented by precipitation, descends through cracks.

TEMPERATURES UNDER THE ICE

It will be seen in figure 3 that the temperature of the surface water under the ice is often considerably above the freezing point of the surface sample for some time after the formation of the ice. This may be due to the thinness of the fresher surface layer early in the winter, which would make the method of taking the observations more likely to cause an admixture of saltier water in the surface sample. Later in the winter the surface water temperature is usually close to the freezing point of the surface sample as is to be expected through its equilibrium with the ice. Surface water temperatures above 0°C. occur at times immediately after the freeze-up and during the winter after thaws. Temperatures above 0°C. usually occur for a considerable period before the disappearance of the ice.

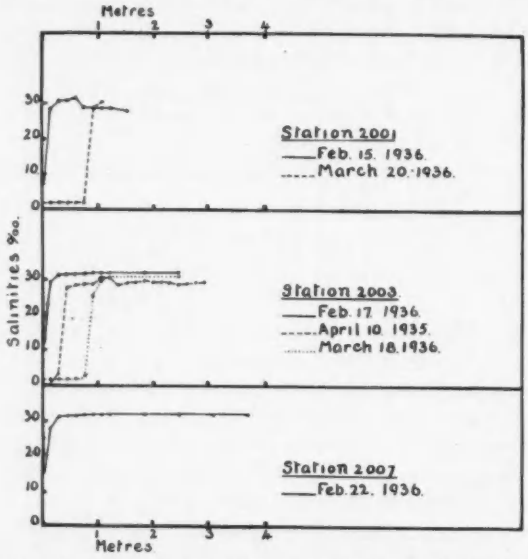

FIGURE 2. Salinities of samples taken at various depths below the ice in Bideford river near the head of tide (Sta. 2001), at the principal observation station (Sta. 2003) and about two and one-half miles farther towards the open (Sta. 2007). No salinities below 1.8 per mille were determined and salinities shown at this value may be lower.

The surface water temperature is determined by the equilibrium of relatively fresh water with ice. It is, therefore, usually well above the freezing point of the more saline bottom water. Cooling of the latter to its freezing point is prevented by the blanket of warmer fresher water. The data indicate that the greatest cooling of the bottom water occurs early in the winter when there is open water either at the observation station or within a relatively short distance. The coldest bottom water is to be expected when some factor such as wind or current prevents the formation of ice in spite of very low air temperatures. Unfortunately, records of the wind and temperatures at the time of ice formation are not sufficiently complete for our data to show a correlation.

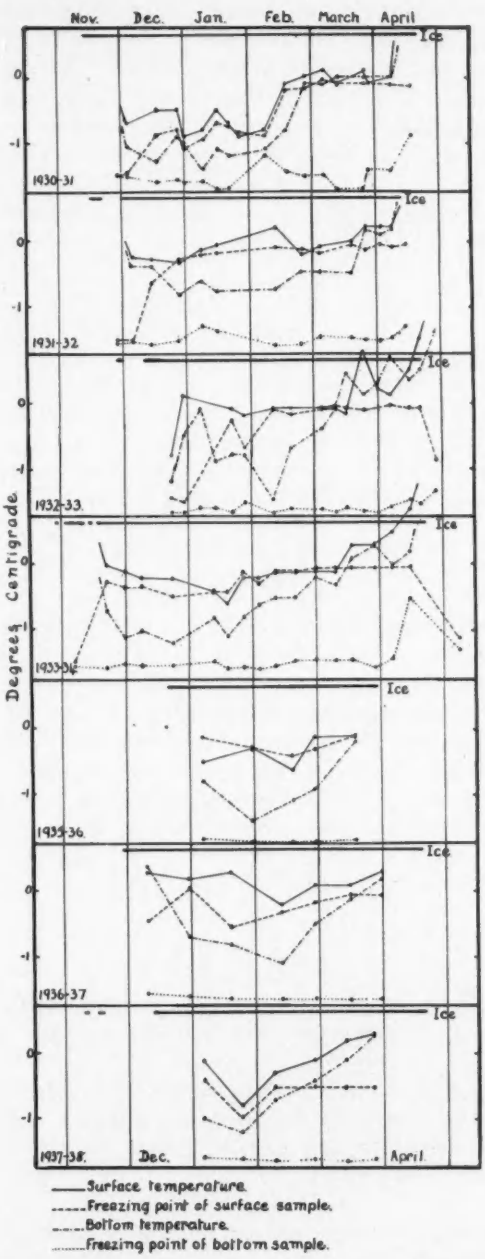

FIGURE 3. Surface and bottom temperatures and freezing points of surface and bottom samples at the principal observation station about one mile below the head of tide in Bideford river.

It will be seen that the temperature of the bottom water actually increases as the winter progresses. This is not caused by warm bottom water entering the inlet, as observations in the middle of Malpeque bay indicate that the bottom water is cold there. Heat to warm the bottom water must come from the bottom or by radiation from above. Petersen (1892) recorded temperatures about 7°C. at a depth of 7.5 cm. (3 in.) below the surface of mud underlying water near its freezing point. The residual heat in the bottom seems a probable source of heat for warming the bottom water. Solar radiation may also contribute especially when the sun is high and the ice free from snow.

DISAPPEARANCE OF THE ICE

From 1931 to 1938 the ice disappeared at the observation station at various dates from about the end of March to late in April. It usually disappears first at the extreme head of the inlet and along the shores, and is accompanied by an inflow of water from rain or melting snow. It seems that the ice is melted from below to a great extent, since the temperature of the underlying water is higher than 0°C. for a considerable time before the disappearance of the ice, as is shown in figure 3.

DISCUSSION

The general picture of conditions under the ice is that of a dynamic system in which, with the water warmer than 0°C. for long periods, temperatures and salinities change considerably during the season, and do not long remain stationary.

There are two salient and related features of the conditions under the ice. The first is the formation of a thin layer of relatively fresh water at the surface. The second is the protection which this thin layer gives to the bottom water by having a minimum temperature considerably above the freezing point of the latter. The presence of ice thus prevents convectional cooling of the bottom waters and even favours a warming of them. This is probably an important factor in the distribution and survival of many organisms.

SUMMARY

Ice may be formed before any considerable proportion of the water reaches its freezing point and may persist for a considerable period after the surface water is warmer than 0°C.

A thin layer of water of very low salinity is formed under the ice soon after its formation and persists until the disappearance of the ice.

The salinity of the bottom water usually remains high throughout the winter with the exception of some reduction during heavy thaws, especially towards the spring.

The minimum possible temperature of the fresher surface layer in the presence of ice is considerably above the freezing point of the bottom water. The latter is thus protected from cooling to a considerable extent. Convectional

mixing becomes difficult after the development of the thin surface layer of lower salinity. The temperature of the bottom water then usually rises as the winter progresses until it approximates that of the surface water.

REFERENCES

NEEDLER, A. W. H. *Bull. Biol. Bd. Can.*, **22**, 1-35, 1931.
PETERSEN, C. G. J. *Rep. Danske Biol. Sta.*, **3**, 31, 1892.
SPARCK, R. *Rep. Danske Biol. Sta.*, **33**, 61-62, 1927.

Ices with Sodium Nitrite and Sodium Acid Phosphate for Fish Preservation

By H. L. A. Tarr and P. A. Sunderland
Pacific Fisheries Experimental Station

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ABSTRACT

Ice containing sodium nitrite plus sodium acid phosphate inhibits bacterial spoilage of dressed chum salmon to approximately the same extent as sodium nitrite ice alone. Intensity of red colour in the cooked muscle of this fish is normally intensified by sodium nitrite.

In a previous communication (Tarr and Sunderland 1940a) it was shown that ice containing sodium nitrite effectively retards bacterial spoilage of dressed halibut, black cod and pink salmon stored therein. Recently it has been found that, within the limits investigated, the more acid the reaction of a bacterial culture medium, or of fish flesh itself, the more pronounced the bacteriostatic activity of sodium nitrite (Tarr and Sunderland 1940b; Tarr 1941). The possibility of enhancing the bacteriostatic action of ice containing sodium nitrite by acidifying it slightly by incorporation of an acid buffer salt suggested itself and was investigated with the following results.

METHODS

Four different ices having the composition given in table I were prepared, the method of preparation being similar to that described for ice containing sodium nitrite (Tarr and Sunderland 1939, 1940a). Hydrogen ion concentration was

Table I. Composition of ices employed

Ice no.	Amount of substance added	Analysis of a solution prepared by melting approximately 2 kg. of the ice after crushing and mixing prior to use	
		pH	$NaNO_2$ in parts per million
1	None (control)	6.5	Not appreciable
2	0.1% $NaNO_2$	6.5	1015
3	0.2% $NaH_2PO_4.H_2O$	5.0	Not appreciable
4	0.1% $NaNO_2$+0.2% $NaH_2PO_4.H_2O$	5.7	910

measured by means of a Beckman pH meter in conjunction with glass electrodes, the pH of the fish muscle itself being determined on the minced tissue after mixing thoroughly with about one-half its weight of water. The number of viable bacteria in the muscle was determined by the technique previously described (Tarr and Bailey 1939; Tarr and Sunderland 1940a). The fish flesh was cooked by placing portions in cold water, bringing the water rapidly to the boil, and boiling for three minutes. The flesh so cooked was used for tasting and also for the determination of colour intensity, using an Armstrong colorimeter (Charnley 1936), an instrument designed specifically for use in the Dominion Canned Salmon

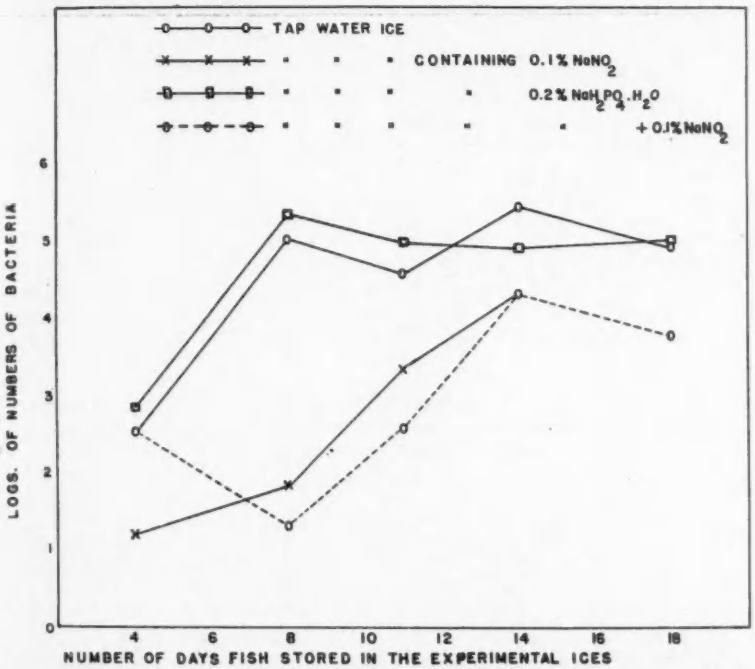

FIGURE 1. Increase in numbers of viable bacteria in chum salmon stored in four different ices.

Inspection Laboratory, Vancouver, B.C., in determining the red and yellow colour in canned salmon, and supplied to us by Mr. Norman L. Armstrong, Laucks Laboratories, Vancouver, B.C. Readings were made to the nearest one-quarter of a Lovibond unit for both red and yellow components, but in view of the fact that the yellow colour was not appreciably altered by the presence of nitrite in the flesh only variations in the red have been recorded.

EXPERIMENTAL

Twenty chum salmon (*Oncorhynchus keta*) weighing from 5 to 8 lb. (about 2 to 3.5 kg.) were obtained from a local fish company. These fish had been dressed, washed and iced on board a fishing boat and were landed 5 to 6 days

after capture. Five fish were iced with each of the four different ices in separate compartments of an insulated box, the ice being replenished with ice of the same type as it melted. At intervals one fish was withdrawn from each compartment and examined.

The results of this experiment are recorded in table II, the number of viable bacteria in the fish after various storage periods being shown graphically in figure 1. It is obvious that the ice containing sodium nitrite alone and that containing sodium acid phosphate (NaH_2PO_4) in addition to sodium nitrite both

TABLE II. Influence of four different

No. of days fish stored in the experimental ices	No. of ice employed (see table I)	Viable bacterial counts in colonies per g. of wet muscle	pH of muscle	$NaNO_2$ in p.p.m. of wet muscle	Colour of cooked mus in red (Lovibond) uni (readings made by tw different individuals	
4	1	326	6.25	0	2.5	2.5
	2	16	6.20	180	2.75	2.75
	3	668	6.20	0	2.75	2.5
	4	332	6.15	96	3.0	3.25
8	1	114,000	6.20	0	2.75	2.75
	2	66	6.25	178	2.75	3.00
	3	220,000	6.30	0	2.5	2.5
	4	20	6.20	186	3.0	3.0
11	1	36,400	6.35	0	2.0	2.25
	2	2,180	6.25	30	3.5	3.5
	3	95,000	6.40	0	2.25	2.25
	4	360	6.25	167	4.0	3.75
14	1	258,000	6.40	0	3.0	3.25
	2	19,600	6.25	175	3.5	3.75
	3	81,000	6.30	0	2.75	2.5
	4	19,800	6.25	157	3.5	4.0
18	1	80,600	6.40	0	3.5	3.5
	2	6,000	6.35	165	3.5	3.75
	3	108,000	6.20	0	3.5	3.5
	4	5,920	6.35	107	3.75	3.5

retarded bacterial development and the accompanying onset of staleness more effectively than did tap-water ice. However, both these ices conferred about the same degree of protection from bacterial spoilage and it would therefore appear that no useful purpose can be served by acidifying ice containing sodium nitrite in this manner. Ice containing sodium acid phosphate alone proved no more effective in preventing bacterial spoilage than did tap-water ice.

The pH of the muscle of the fish stored in the ices containing sodium acid phosphate was not appreciably different from that of fish stored in ices without

this salt; the small variations encountered are probably accounted for by differences in individual fish and the slow increase in basic substances due to bacterial multiplication. This finding bears out the contention made elsewhere by the writer, namely that the pH of fish muscle can only be altered by rather severe or prolonged treatment with acidic or basic substances; in this experiment the pH of the fish muscle was itself sufficiently low to permit the marked bacteriostatic activity of the nitrite-containing ices (Tarr 1941).

In no case did the amount of sodium nitrite in the muscle of fish stored in ice

he keeping quality of chum salmon

Organoleptic tests
xternal appearance of fish from all four ices good, no unpleasant odour from poke region. o difference in odour of cut muscle. Nitrite-containing ices caused a faint, but by no means arked, brown discoloration along cut surfaces of nape and poke, and slight browning of ood clots due to methaemoglobin formation.
sh from ices 1 and 3 becoming stale with a dirty yellowish bacterial slime appearing on their rfaces. External appearance of fish from ices 2 and 4 definitely superior: no bacterial slime ident, surfaces bright and pokes gave off no unpleasant odour. No difference in taste of oked flesh noticed.
sh from ices 1 and 3 stale and slimy, pokes smelled very sour, cut muscle gave off stale odour. sh from ices 2 and 4 showed no bacterial surface slime, and gave off no stale odour, cut flesh m and no unpleasant odour. Little difference noticed in cooked flesh, possibly slight bias favour of fish from ices 2 and 4.
sh had very much the same general external appearance as after 11 days. Cooked flesh m fish from ice 1 was stale, from ice 3 slightly stale, and from ices 2 and 4 still edible and t stale though not as tasty as fresh fish.
xternally, fish from all four ices definitely stale, though those from ices 1 and 3 were more my and putrid that those from ices 2 and 4. Cut flesh from fish from ices 1 and 3 very stale th hydrogen sulphide-like odour, that from fish from ices 2 and 4 not unpleasant and not ale. Cooked flesh of fish from ice 1 was stale and unpleasant, from ice 3 very stale and ite inedible, from ice 2 edible but rather tasteless and from ice 4 edible and of fairly good vour.

containing sodium nitrite exceed 200 parts per million, and in many cases considerably less was present. The intensity of red colour in the cooked muscle of the salmon which had been exposed to sodium nitrite was usually greater, and in no instance less, than that of fish stored in nitrite-free ices. The expectancy that sodium nitrite treatment will cause an increase in intensity of red colour in cooked chum salmon muscle may therefore be said to be high. Such factors as the variation in red colour in individual fish, and also the amount of haemoglobin capable of being converted to nitrosohaemoglobin (and on heat treatment possibly to

nitrosohaemochromogen: White, Cook and Winkler 1940), probably play an important part in determining the intensity of red colour developed.

ACKNOWLEDGMENT

The writers are indebted to the Canadian Fish and Cold Storage Company, Prince Rupert, and especially to Mr. J. E. Boddie and Mr. H. Worsfold of that Company, for providing the facilities for preparing and crushing the ices used.

REFERENCES

CHARNLEY, F. *Biol. Bd. Can. Prog. Rep. Pac.*, **29**, 12-16, 1936.
TARR, H. L. A. *J. Fish. Res. Bd. Can.* **5** (3), 265-275, 1941.
TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.
TARR, H. L. A., AND P. A. SUNDERLAND. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **41**, 15-16, 1939.
J. Fish. Res. Bd. Can., **5** (1) 36-42, 1940a.
Fish. Res. Bd. Can. Prog. Rep. Pac., **44**, 16-17, 1940b.
WHITE, W. H., W. H. COOK AND C. A. WINKLER. *Canad. J. Res.*, **18D**, 260-265, 1940.

Studies on Salt Fish

IV. Survival of *Eberthella typhosa* and *Escherichia coli* on Salt Fish

By M. Frank and E. Hess
Atlantic Fisheries Experimental Station

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ABSTRACT

Eb. typhosa and *Es. coli* survive on salt fish agar and in salt fish broth at 5 to 6° C. for periods of three months and more. On salt fish itself survival is reduced, particularly for the former organism. Survival is favoured by low storage temperatures and heavy contamination.

INTRODUCTION

Raw fish consumption, where it is practised, has led in many cases to typhoid outbreaks (Damon 1928). While there are no recorded instances of similar occurrences with salt-cured fish, the survival powers of *Eberthella typhosa* in high salt environments (Kenyoun 1925, Tanner 1919) present the problem of a potential spreading of this organism by means of such a medium. Our aim has been to determine whether *Eb. typhosa* would survive on the surface of salt-cured cod while under commercial storage conditions (5 to 6°C.) prior to drying. Contamination through polluted sea-water during the washing of the fish, or through direct contact with carriers was considered as a possible source of infection.

Abundant evidence exists (Kenyoun 1925, Tanner 1919) proving the continued survival of *Eb. typhosa* as well as *Es. coli* under conditions of high salt environment (saturated brines). Karaffa-Korbutt (1912) recommended that in pickled meat and fish over 10 per cent of salt be included to avoid growth of the common organisms concerned in gastro-enteric disease. Salt concentrations above 10 per cent were found to be inhibitory but not lethal.

In order to evaluate the possible use of standard *Es. coli* tests as an index of sanitary control in the salt-curing of fish, the relative survival powers of *Eb. typhosa* and *Es. coli* will be considered, taking into account temperature of storage, degree of contamination and salt concentration.

EXPERIMENTAL

Degree of Contamination

Salt fish blocks (½×2×3 inches or 1.3×5.1×7.6 cm.) in duplicate were held at 5 to 6° C., following artificial contamination by means of a 15 minutes'

immersion in a sea-water bath containing high and low concentrations of the test organisms, cultured previously on agar slants at 37° C. for 18 to 24 hours, the concentration being determined nephelometrically.

Direct qualitative samplings of less than 1-gram size from the cut surface of the salt fish blocks were transferred, in the case of *Eb. typhosa* tests, to a tetrathionate enrichment broth, and positive cultures confirmed on eosin-methylene blue plates, in lactose and glucose broth, lead-acetate agar, and checked for motility and morphology. Samples for *Es. coli* were seeded at 2-day intervals up to 36 days, and then at approximately 5-day intervals into brilliant-green lactose bile or lactose broth, and confirmed on eosin-methylene blue plates.

Occasional indol, methyl red, Voges-Proskauer and citrate tests were added. At the first occurrence of negative results on successive sampling, the findings were checked in triplicate before the recovery of *Eb. typhosa* or *Es. coli*, respectively, was considered impossible.

TABLE I. Survival time of the two species on salt fish in days, the degree of contamination being expressed as numbers of organisms per ml. of the sea-water used for immersion.

Species	*Eberthella typhosa*		*Escherichia coli*	
Contamination (no. per ml. of sea-water)	18,300	100	40,000	100
Storage temp. (° C.)				
6	>22, <50	<22	>72	>48, <54
25	—	—	>25, <27	>10, <12
37	—	—	>10, <12	>3, <5

The results, as shown in tables I and II, indicate with one exception that in case of heavy contamination the chances for survival of both *Eb. typhosa* and *Es. coli* are increased considerably. The fact that the pH of the salt fish muscle had an average value of 7.6 may help to account for the low bactericidal value of the sodium chloride. Goshorn, Degering and Tetrault (1938) pointed out that as far as the bacteriostatic action of sodium chloride solution on *Es. coli* is concerned, only high concentrations are effective and then only in the range in pH from 5 to 6.

TEMPERATURE OF STORAGE

In the case of the *Es. coli*-contaminated salt fish blocks, tests were made during storage not only at 6° but also at 25° and 37° C. The results clearly indicate increased chance of survival with a lowering of the storage temperature (table I). This may be due partly to the absence of growth of salt-tolerant organisms at the lower temperature, in contrast to 25° and 37° C., where an abundant population of spore-formers and micrococci developed, accompanied by definite spoilage of the fish.

Salt Effect

In order to gain a picture of the survival rate of the test organisms at high salt concentrations, quantitative recovery counts of the test organisms were made in duplicate from salt-free fish agar and fish agar containing 25 per cent sodium chloride, as well as from salt-free fish broth and fish broth containing 15 per cent sodium chloride.

The broth was obtained by extracting 1,000 g. fresh cod fillets in 1,000 ml. distilled water overnight in the cold, filtering, adding 15 per cent salt to one portion, and sterilizing. Agar was added to another portion of the fish extract,

Table II. Percentage survival of *Eb. typhosa* and *Es. coli* in salt-free and salt media at 6° C.

	Salt-free agar		25 per cent NaCl agar	
Eb. typhosa Initial no. of organisms	8.1×10^{7}	3×10^{4}	2.76×10^{8}	6.3×10^{4}
Survival				
After 8 days	62%	10%	15%	10%
" 36 days	62%	5%	—	0%
" 92 days	26%	0%	—	0%
Es. coli Initial no. of organisms	1×10^{9}	4.2×10^{4}	1.4×10^{9}	2.4×10^{4}
Survival				
After 14 days	100%	83%	14.3%	58%
" 91 days	25%	1%	0.4%	16%
Eb. typhosa	Salt-free fish broth		15 per cent NaCl broth	
Initial no. of organisms	3×10^{8}			5×10^{6}
Survival				
After 10 days	50%			30%
" 94 days	10%			1%

and half of this lot received 25 per cent salt. Counts were done on nutrient agar plates made in duplicate directly from adequate dilutions of the broth cultures, or from the agar culture surfaces by washing the latter quantitatively with a suspension of sterile sand in a sterile 0.85 per cent saline solution.

The results presented in table II show that, although percentage survival was lower in the salt media for both organisms, high salt concentrations did not kill all the bacteria at 6° C. even after the lapse of three months.

DISCUSSION

Es. coli was found to survive longer on the salt fish surface than *Eb. typhosa*, practically independent of the degree of contamination, at least at 6° C. The

presence of *Es. coli* on salt fish, while not necessarily meaning presence of viable *Eb. typhosa* organisms if the fish have been in store at 6° C. for some two months at least, does indicate previous contamination and potential danger, however. When making *Es. coli* tests as sanitary control measures, these points should be taken into consideration.

SUMMARY

Eberthella typhosa was recovered from salt fish blocks after 22 days at 5 to 6° C. and from salt media after periods as long as 94 days.

Escherichia coli was found on salt fish blocks at 5 to 6° C. after 72 days, and on salt media after 91 days.

Survival is favoured by low storage temperatures and heavy contamination, as found by Ballantyne (1930).

The use of tests for *Es. coli* as a measure of sanitary control of salt fish in storage before drying appears justified, when the storage period is known.

REFERENCES

BALLANTYNE, E. N. *J. Bact.*, **19**, 301-320, 1930.

DAMON, S. R. Food infections and food intoxications, 1-266, Williams and Wilkins, Baltimore, 1928.

GOSHORN, R. H., E. F. DEGERING AND P. A. TETRAULT. *Ind. Eng. Chem.*, **30**, 646-648, 1938.

KARAFFA-KORBUTT, K. *Z. Hyg. Infektionskrank.*, **71**, 161-170, 1912.

KENYOUN, C. *U.S. Publ. Health Rep.*, **40**, 819-823, 1925.

TANNER, F. W. Bacteriology and mycology of foods, 1-592, Wiley & Sons, New York, 1919.

The Influence of Temperature and Salinity on the Condition of Oysters (*Ostrea virginica*)

By J. C. Medcof and A. W. H. Needler
Prince Edward Island Biological Station

(*Received for publication February 15, 1941*)

ABSTRACT

Below 5° C. oysters do not change in fatness, as judged by the ratio, dry weight to space between the valves. Between 5 and 15° they get thin. Between 15 and 20° they fatten. Above 20° they may fatten slightly but spawning makes them thin. When the salinity drops below 20‰ no fattening takes place even at temperatures between 15 and 20°.

INTRODUCTION

When oyster meats are creamy white, firm, plump and seem to fill the space between the shells almost completely they are said to be fat or in good condition. Thin meats or those in poor condition are usually darker coloured or semi-transparent, flaccid, with a high water content and seem to occupy only part of the shell cavity, the rest of which is filled with shell liquor. It has been frequently illustrated through chemical analysis (Tully 1936) that the condition of oyster meats influences their nutritive value and it is well known to the trade that it influences their market value. It is therefore important that the oyster farming industry should be able to produce fat oysters.

MATERIAL AND METHODS

After a preliminary study the following value was adopted as the "index of condition":

$$1000 \times \frac{\text{Dry weight of the oyster meat in grams}}{\text{Volume of the space between the valves in millilitres}}$$

After a thorough cleaning and scrubbing, the oysters were opened with an ordinary oyster knife. The shells were first pried apart slightly and the shell liquor (which was discarded) allowed to drain out before cutting into the adductor muscle or any other part of the body. The whole of the oyster meat, including whatever body fluids were released in the process, was then cut free into a weighed evaporating dish and dried to constant weight at the temperature of boiling water. The dry weight was measured to the nearest 0.1 g.

The volume of the space between the shells was determined, using the overflow method, by weighing the water displaced by first the entire oyster and then the empty shells. In order to standardize the wetness the oysters or shells were immersed in water and allowed to drain for three minutes before volume determination.

In 1939 the index was determined separately for each oyster and the average value for ten taken as representative for each sample. In 1940 to reduce the routine work the tests were made on two groups of five oysters each and the average of the two results taken as representative.

Seasonal changes in the value of the index were followed by systematic sampling of several stocks of oysters. At the same time water temperatures and salinities were recorded and spawning activity observed by examination of either gonads or plankton. The temperatures and salinities recorded (figure 1) are averages of surface and five-foot (1.5 m.) depth readings and each date on which spawning took place is indicated by "S". Only the results from Malagawatch and Gillis cove, both in the Bras d'Or lakes, N.S., and from two beds in Shediac bay, N.B., are presented here. Similar data from the Cooper bed in Malpeque bay, P.E.I., South gut in St. Ann bay, N.S., and Stoney point in the Bras d'Or lakes are in accord with the conclusions.

It was found that the condition of oysters may vary with size, shell shape and the average depth of the water from which they are fished. For this reason an effort was always made to select for the tests only round "cup" oysters measuring between 9 and 13 cm. in the greatest diameter of the shell. The areas from which the oysters were fished were also marked by careful ranges on shore or by buoys.

In 1940 the supply of oysters on the Gillis cove plot was exhausted. Before this happened a new area was selected close to the old and for a while samples were taken from both the old and new plots.

INFLUENCE OF TEMPERATURE

In the winter when the water temperatures are below 5° C. and the oysters hibernating, there is little change in condition. The data show that at the Cooper bed in Malpeque bay the index was 114 on December 7, 1939, 115 on February 23, 1940, and had fallen to 110 by May 10. At Malagawatch between December 15, 1939, and May 11, 1940, the index fell from 102 to 98. In both these areas the water temperatures had fallen below 5° by December 7, 1939, but had risen above 5° before May 11, 1940. From these data it may be inferred that during the hibernation period, when it is colder than 5°, there is no change in the condition whatever.

As the temperature rises in the spring above 5° the index falls more and more rapidly until the temperature reaches 15°. This phase is well illustrated in the 1940 records for Malagawatch (middle of figure). Then, above 15° and until 20° is reached, there is usually a rapid rise in the index and at this time the spawn develops. This phase is clearly illustrated in the results for Shediac bay (top of figure). When the temperature rises sharply above 20° spawning

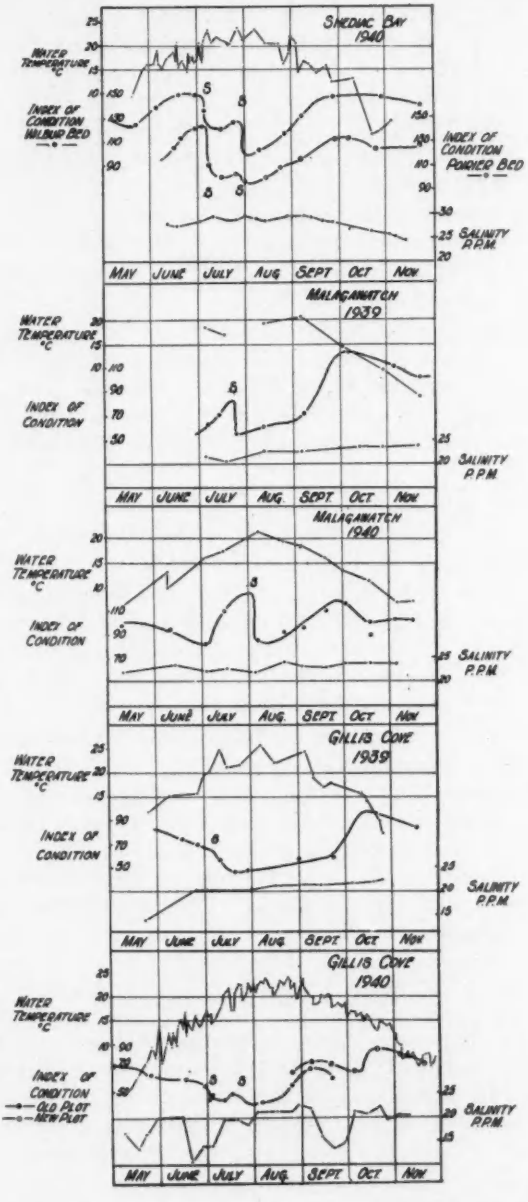

FIGURE 1. Changes in water temperatures and salinities and in index of condition of oysters.

usually occurs with a sudden decrease in the index as is also well shown in the Shediac bay results. If the temperature continues above 20° after spawning there may be a slight rise in the index, but the condition does not improve rapidly until the temperature falls below that level and most of the fattening of late summer and autumn occurs between 20 and 15°, as is particularly well shown in the curves for Malagawatch. This change in the index is presumably associated with glycogen storage.

When the temperature falls below 15°, the index also falls, as is shown by the Malagawatch results, until the temperature reaches 5°. When it goes below 5°, the decrease in the index nearly or altogether ceases and the annual cycle is completed.

To summarize the relation of temperature to changes in condition: below 5° there is little change; between 5 and 15° the index decreases; between 15 and 20° it usually increases; above 20° it may increase slowly but decreases sharply at spawning. This generalization from the data was made use of in the interpolation necessary for drawing the smooth index of condition curves in the figure.

Orton (1928) has already detected a temperature band favourable to fattening in *O. edulis*. Approximately this extends from 10° to 15° and apparently corresponds to the 15° to 20° range just described for *O. virginica*.

Galtsoff's results (1928, figure 8) indicate that water pumping, which is a measure of feeding activity, begins in *O. virginica* at 5° and that the rate increases slowly up to about 10°. From 10 to 15° there is practically no change in pumping rate. Above 15° there is a rapid increase in rate up to a level not clearly marked but apparently between 20 and 23°. Above this level the pumping rate falls off.

Thus the relation of change in condition to temperature may be explained on the basis of the balance between anabolism, resulting from the intake of food, and catabolism. Below 5° there is no feeding but there is likely to be practically no catabolism and the index does not change. Between 5 and 15° it is probable that feeding, which is not extensive, is insufficient to offset catabolism since the animal grows thin. Between 15 and 20° food collection goes on at a high rate and the animals fatten. The data are insufficient to permit an analysis of changes above 20°.

INFLUENCE OF SALINITY

The data for Gillis cove (lower part of figure) illustrate a complication of the relations just described. There was no spring rise in condition either in 1939 or 1940 and the autumn rise in 1940 was interrupted. Water temperatures at these times favoured normal fattening for they ranged between 15 and 20°. The salinity of the water, however, was low,—20‰ or lower.

Hopkins' (1936) experiments with *O. gigas* have shown that water pumping (feeding) rate becomes very irregular when the salinity drops below 20‰. If *O. virginica* behaves in the same way the decline in the index at low salinities can be ascribed to poor feeding resulting in starvation.

INFLUENCE OF SPAWNING

Spawning results in a decrease in the index, as is best illustrated in the results for the Poirier bed, Shediac bay. A heavy spawning between July 7 and 8 produced a drop in the index from 142 on July 2 to 110 on July 9. In contrast to this conspicuous change is that for Gillis cove where the oysters produce only meagre amounts of spawn. Spawning in 1940 took place between June 30 and July 2, and produced a drop from 60 on June 14 to 47 on July 3.

REFERENCES

GALTSOFF, P. S. *Bull. U.S. Bur. Fish.*, **44**, 1-39, 1928.
HOPKINS, A. E. *Ibid*, **48**, 345-364, 1936.
ORTON, J. H. *J. Mar. Biol. Assoc.*, N.S., **15**(2), 365-427, 1928.
TULLY, J. P. *J. Biol. Bd. Can.*, **2**, 477-484, 1936.

Migrating Behaviour of Sea-Running *Salvelinus fontinalis*

By H. C. White
Fisheries Research Board of Canada

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ABSTRACT

This trout in Moser river, Nova Scotia, descends to the sea in April and May, with risin. temperature. Falling temperature lessens or stops descent, causing some to return upstream The smallest showed straying in 1940 to other streams several miles distant and smaller percentage return to river than for other sizes. Return was related to freshets in the main river, rather than in tributary of origin. Homing to latter was definite, but only partial, and apparently lessened by winter sojourn elsewhere. Period of absence in sea ranged from 42 to 84 days.

Observations made in 1940 on the sea trout (*Salvelinus fontinalis*) of Moser river, Nova Scotia, were largely confirmatory of those previously reported (White 1940), but tagging gave definite records of the movements, growth, etc. of individual fish.

THE SEAWARD RUN

The barrier and traps on Mill brook (figure 1), the branch where the marking and tagging has been done, were in operation on April 17, sixteen days earlier than in 1939. There had undoubtedly been some movement of trout in the brook before the placing of the traps, but the earlier operation gave a better record of the downstream migration than was previously obtained.

Between April 17 and June 9, 1,455 trout were taken. These consisted of (1) 1,196 of smolt size, (2) 141 adults showing various stages of acquiring the silvery coat of guanin, and 118 fish which showed no guanin, and were either small individuals or definite stream types. In figure 2 the descending columns show part of the downstream run and the broken line the water temperatures. The correlation between this run and temperature is more definite than was found for the run of the previous year.

MARKING AND TAGGING

As in 1939, the descending trout were marked by shaving off the adipose fin, but this year 113 of the descending trout were tagged with small consecutively numbered celluloid discs attached to the anterior base of the dorsal fin. Discs one-quarter inch (6.3 mm.) in diameter were used for the larger trout and were attached with twenty gauge nickel wire. For tagging the smaller trout smolts, discs of one-eighth inch (3.2 mm.) diameter were attached with very small silver wire. These tags were so small and light that they caused little, if any, inter-

ference with the normal behaviour of the fish. That the handling necessary for the attachment of the tags, or the tags themselves did not affect the trout adversely was indicated in the recaptures of the tagged trout by anglers. Several trout on which tags were placed in the forenoon were taken by angling on the afternoon of the same day and a number of them were caught the day following the tagging.

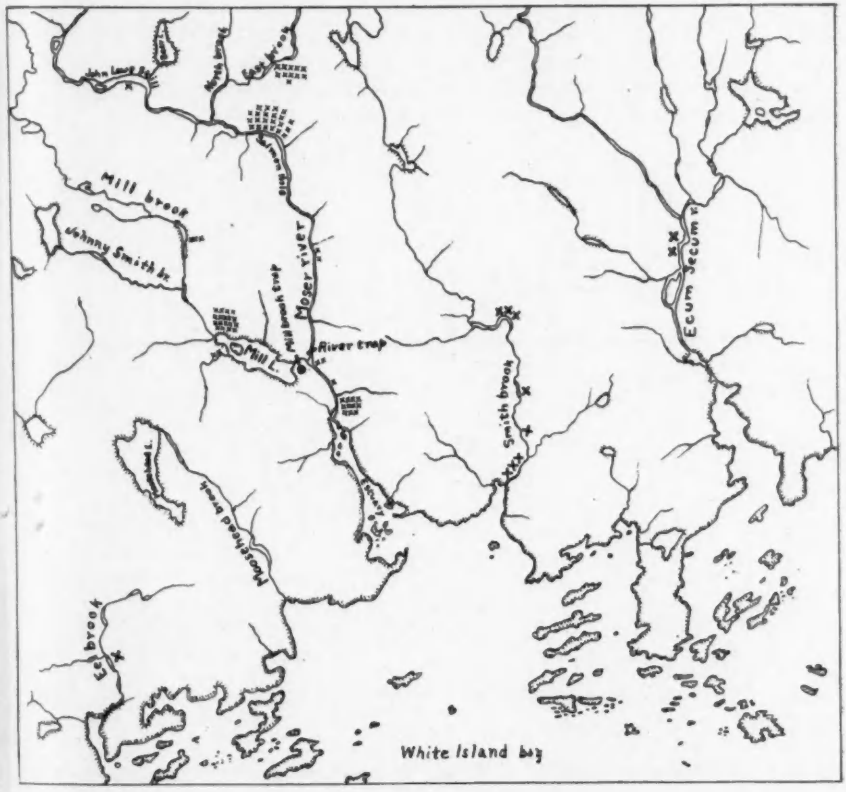

FIGURE 1. Map of Moser River district, showing recaptures (exclusive of recaptures in traps) of trout marked or tagged at Mill brook trap (solid circle). Large crosses indicate those taken in other waters, small crosses those in the Moser river system.

LOITERING AND REVERSING

Some of the tagged trout after descending through the trap ascended to Mill lake again where they remained from 1 to 8 days. This behaviour occurred mostly during April when the water temperature was below 5° C., but also with higher temperatures in May. In figure 2 the extent of this return movement is shown by the heights of the columns above the base line, and the downstream

run, included for comparison, is indicated on a different scale below the base line. A study of this figure will reveal that when the downstream run decreases during a drop in temperature the upstream run increases. Thus it seems that low temperature, and particularly a drop in temperature, not only retards the tendency to migrate down stream but causes a reversal of the movement among fish which have already started on the downstream migration.

Also, some trout, instead of descending directly to the sea, loitered in the river as shown by the capture there of two fish, 7 and 16 days after being tagged.

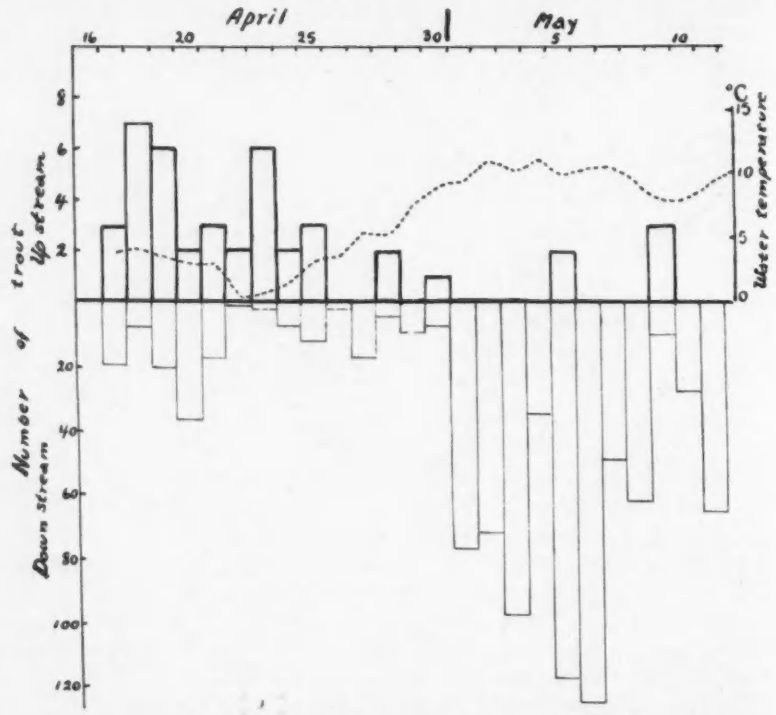

FIGURE 2. Downward migration of trout (descending columns) and reverse migration (ascending columns) during the spring of 1940 in Mill brook. The water temperature (taken 9 a.m. daily) is indicated by the broken line.

However, 80 per cent of the recaptures during descent of the river were made from a few hours to 3 days after tagging. When the water was low in the river the seaward migrants were readily taken by anglers, but during even moderately high water few were caught in the river though taken more readily in the upper estuary.

STRAYING TO OTHER STREAMS

After their descent to the sea only a few recaptures of the tagged trout were recorded before the return run from the sea. Two of these were taken in mid-

June in Smith brook which is some four miles from the traps but less than a mile from the mouth of the Moser river estuary (figure 1). These fish were taken in the brook at the extreme head of tide water before the general upstream migration of the trout of that brook. Their presence was coincident with the spawning there of large numbers of smelts and the trout were feeding upon the abundant supply of eggs. During this spawning large numbers of eels, also from the sea, were attracted into the brook. Since the sea trout are known to be attracted by the washings from canning factories or fish-cleaning wharves it is not unlikely that these particular fish were attracted there by the abundant supply of smelt eggs. During the summer 95 trout were taken from one-half to three miles (1 to 5 km.) up this brook and 5 of these, or 5.3%, were trout which had been

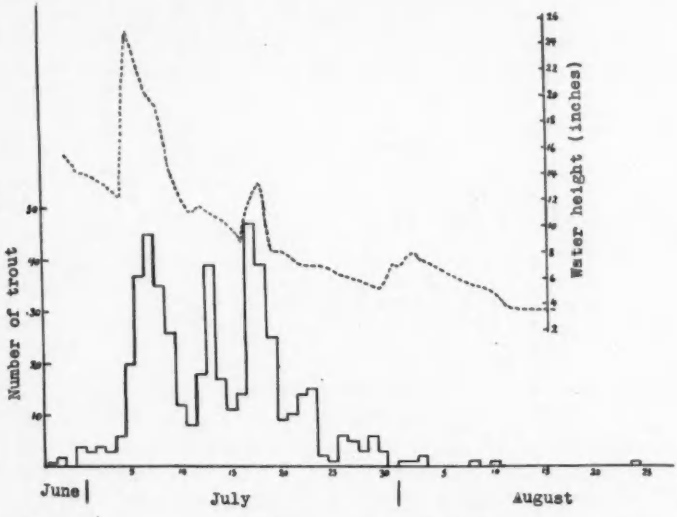

FIGURE 3. Numbers of trout taken in the river trap during the return migration, in relation to height of water in the river.

marked as smolts during their descent of Mill brook. Since Mill brook trout constitute only a minor proportion of the trout from the Moser river, it is probable that Moser river trout form a fairly high percentage of the trout of Smith brook. Two tagged trout were taken in Ecum Secum river nearly eight miles (13 km.) to the eastward of Moser river and a marked trout was taken in Eel brook more than four miles (6½ km.) to the westward (figure 1). These records indicate a fairly wide wandering from Moser river.

RETURN MIGRATION

The first trout taken in the traps on return from the sea was in the Mill brook trap on June 20; but during the whole of June only 5 were taken in this trap and 7 in the river trap. As in the previous year, the run was almost confined

to the month of July, 75 per cent of the trout entering the traps being taken in the period July 5 to 19. During the entire migration 321 were taken in Mill brook and 497 in the river. In 1939, the runs up Mill brook and the river were 86 and 602 respectively.

In our previous paper (White 1940) we have stated that the low percentage of fish ascending the brook rather than the river was "as if the brook were becoming less favourable for the trout". In 1940 with lower temperatures and more water in the brook a much higher percentage of the trout ascended the brook.

In figure 3, which shows the daily catches of trout in the river trap and also the daily water levels of the river, it will be seen that the peaks in the run are definitely associated with rises in the water level. In figure 4, which gives similar data for the brook, it will be noted that only the main peak is associated with a definite rise in the brook water. The other peaks, however, are related to rises in the river and the runs of trout into the river. Rain which causes a

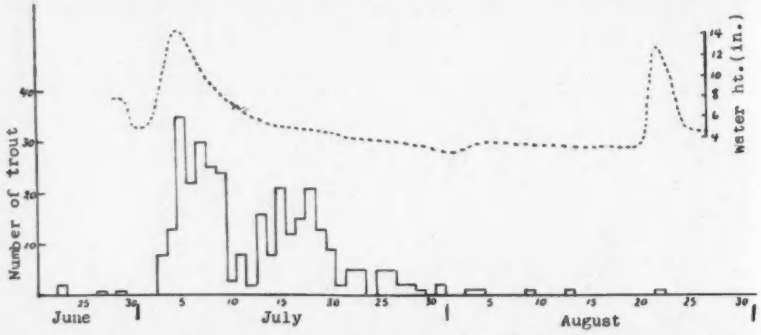

FIGURE 4. Numbers of trout taken in Mill brook trap during the return migration, in relation to height of water in the brook.

definite rise in the river may not perceptibly influence the height of the brook, which is the outlet of Mill lake, and yet give a run of trout in the brook.

In 1939, of the marked trout from Mill brook that returned to the river, only 24.4% ascended Mill brook. In 1940, better water conditions prevailed in the brook, and 83% of the returning trout which were tagged at Mill brook ascended the brook. The rest of the tagged fish, namely 17%, were taken ascending the river and on the same dates that other trout ascended Mill brook, indicating that their ascent at this time of the river rather than the brook was not caused by unfavourable conditions in the brook.

Of all the Mill-marked fish (marked in both 1939 and 1940 by clipping the adipose fin) that returned from the sea in 1940, 70.7% entered the Mill brook trap. This is some 12% lower than for the trout tagged in 1940. Since all were originally Mill brook fish, the difference may be accounted for by the acclimating of many of the Mill-marked fish to the river after their ascent in 1939 when the brook was unfavourable. While some of these marked trout descended the river and ascended the brook in the fall of 1939, most of them remained up the

river, as substantiated by the finding in mid-April, 1940, of a number of trout of the 1939 Mill-marking among the catch of poachers fishing through the ice in the Salmon hole, which is some four miles up the river. Five of the 25 trout in their catch were Mill-marked trout.

Trout marked during their descent of Mill brook, and which went to sea and on return ascended the river, have been taken in the Salmon hole and in John Lowe still about a mile (1.6 km.) above, but this year many of them were found in the still waters of East brook, a small branch of North brook which enters the main river a short distance above the Salmon hole (figure 1).

TIME IN THE SEA

In our previous paper (White 1940), we stated from general observations that the trout spent two months in the sea before returning to fresh water. Our

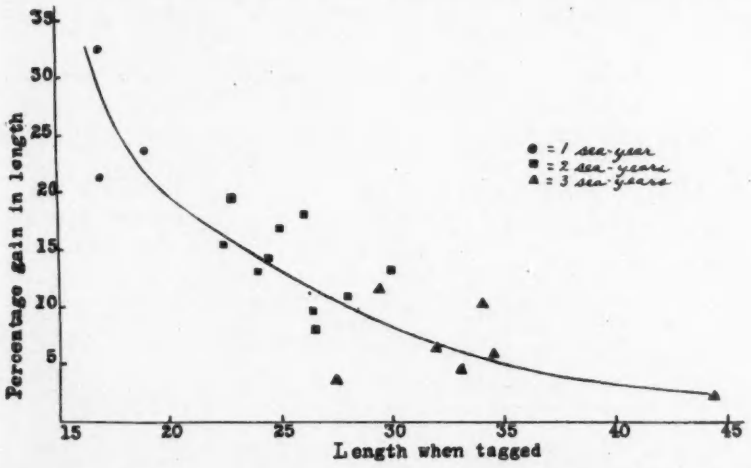

FIGURE 5. Relation of percentage increase in length of trout of different sizes to length when tagged and "sea-age".

definite records of the tagged fish this year show that the periods between their downstream liberation and their recapture on return from the sea range from 42 to 84 days with an average of 64½ days. Those descending as smolts remained away an average of 71 days—one-sea-year fish averaged 64.4 days and two-sea-year fish or older, 59 days. Although, as we have stated above, there was a tendency for some of the fish to loiter in the river during descent to the sea, it appears that, even allowing for this delay, the first downstream migrants spent longer periods in the sea than those descending later. A notable exception to this was the last fish which was tagged, an old male, which returned August 24, almost a month after the run of the other fish. This trout had also the third longest absence.

GROWTH IN THE SEA

From the measurements of the tagged fish before and after their sojourn in the sea, we have been able to ascertain the gains in length made by the individual fish. Increases in length range from 1 to 5.5 cm., with an average of 3.7 cm. Percentage gains in length range from 2.2% to 32.4%. It was found by determining the ages of the fish from the scales, that the younger fish made not only greater percentage, but also greater actual, gains in length. In figure 5 increase in length has been plotted against length when tagged, and the sea-ages of the individual fish are indicated. It is evident that better growth is made by the smaller and younger fish.

SURVIVAL OF TROUT

We tagged 47 smolts ranging from 15 to 20.5 cm., and got a return of only four or 8.5%. Of the medium-sized trout, those ranging from 22 to 28 cm., there was a return of 35.3% and of the large fish, those from 29 to 45 cm., a return of 31%.

The known initial losses due to angling in the river during the descent of the tagged trout were: for the smolt size 2%; medium size 8.8%; and large fish 23%. The taking of the large trout during their descent in the spring is a questionable practice, as at that time they are in poor condition and of inferior quality, while two months later a high percentage of them return as larger prime fat fish.

Of the trout reaching the sea, i.e. after deducting the initial loss from angling, 8.7% of the smolt class, 38.7% of the medium size and 40% of the large fish returned to the traps. A large part of the loss, especially among the small fish, is, as our recaptures have indicated, due to their straying to other streams, but it is probable as another factor that loss from predation is greater among the small fish than among the large.

There was a return to the traps of 18.5% of all the tagged trout, but this does not indicate such a return for the entire run of seaward migrants, since a relatively high percentage of large trout were tagged and returns from these were comparatively high.

REFERENCES

WHITE, H. C. *J. Fish. Res. Bd. Can.*, **5** (2), 176-186, 1940.

The Action of Nitrites on Bacteria

By H. L. A. Tarr
Pacific Fisheries Experimental Station

(*Received for publication December 26, 1940*)

ABSTRACT

Inhibition of the growth of bacteria by sodium nitrite is largely dependent on the pH of the substrate upon which they are cultivated. Thus at pH 6.0, 0.02 per cent of sodium nitrite may entirely inhibit or strongly retard growth, while at pH 7.0 little or no inhibition is in evidence. There is a marked variation in the susceptibility of different bacteria to nitrite, and there appears to be no definite relationship between its effect in retarding growth and in inhibiting aerobic respiration.

The action of nitrites on bacteria has been studied to only a limited extent, and has been mainly, but not entirely, concerned with their influence on organisms associated with meat-curing processes. Lewis and Moran (1928) found that the putrefaction of minced beef in experimental brines by the natural mixed bacterial flora was largely prevented by the addition of 0.03 to 1.5 per cent of sodium nitrite. Their work, as well as that of Tanner and Evans (1934), indicates that strictly anaerobic bacteria of the genus *Clostridium* will tolerate much greater concentrations of sodium nitrite than will the mixed bacterial flora of brines. Both Stephenson (1939, p. 51) and Landerkin (1940) have observed that the growth of different bacteria is inhibited by 0.4 per cent of sodium nitrite. It is noteworthy in this connection that the possible effect of pH on the bacteriostatic action of nitrites apparently had been entirely disregarded until the publication of a brief account of certain collaborative experiments carried out at this Station (Tarr and Sunderland 1940c).

Recent investigations have shown that ice containing sodium nitrite retards bacterial spoilage of dressed fish much more markedly than does tap water ice (Tarr and Sunderland 1939c, 1940a), and that the onset of staleness in fillets intended for fresh, frozen or smoked fish markets is markedly delayed by a brief immersion in a sodium chloride brine containing sodium nitrite (Tarr 1940a, Tarr and Sunderland 1939a, 1939b and 1940b). The present paper records in detail the results of experiments conducted in order to determine some of the conditions under which nitrites inhibit bacterial growth.

EXPERIMENTAL

The cultures employed were those used in previous work (Tarr 1939, 1940b). Solutions of sodium nitrite were prepared from pure sodium nitrite after re-

crystallization from water, and were sterilized by passing them through a Seitz filter. To determine pH, a Beckman meter was used in conjunction with glass electrodes, readings being made to the nearest 0.05 pH unit. Direct counts of bacterial suspensions or cultures were made by means of a Petroff-Hausser counting chamber, motile organisms being rendered non-motile by exposure to chloroform. In the case of several species of *Micrococcus* studied, small clumps of bacteria formed in which individual organisms could not be readily separated, and in which it was impossible to determine with any degree of accuracy the number of single organisms present. For the sake of simplicity in making purely comparative counts such as are recorded in this paper, these clumps have been counted as single organisms. All cultures were incubated at 25° C., manometric experiments being conducted at 30° C.

In view of the fact that it was not considered practicable to study intensively conditions that would allow the incorporation of more than 0.02 per cent (0.00345 M) sodium nitrite in foods intended for human consumption, sodium nitrite concentrations of this order have been used in most of the experiments recorded in this paper. Although experiments have shown that higher concentrations of sodium nitrite (0.01 to 0.1 M) are undoubtedly much more toxic to bacteria, the results have not, for the sake of brevity, been included.

Influence of pH on Inhibition of Growth by Nitrite

in culture media

The medium used to determine the effect of pH on nitrite inhibition was prepared as follows. One and one-half millilitres of sterile Bacto nutrient broth (double strength), 0.5 ml. of 0.2 M phosphate buffer (different pH values), and 0.5 ml. of 0.12 per cent sodium nitrite solution (water in control experiments), were added to 12 x 150-mm. culture tubes. Each tube was then inoculated with 0.5 ml. of a saline suspension of a 16- to 24-hour nutrient agar culture of the organism being studied, the number of organisms present in the inoculum being determined. Counts of the number of organisms present in the inoculated media were made at intervals in order to ascertain to what extent nitrite inhibited growth.

In one experiment (table I) media adjusted to six different pH values between pH 6 and 7.35 were inoculated with two types of bacteria, one of which was known to be sensitive, and the other insensitive, to sodium nitrite. The results, given in table I, show that the growth of one of these organisms (culture 4) was not appreciably affected at any pH by 0.02 per cent of sodium nitrite. On the other hand, in the case of culture 17, growth was strongly inhibited by sodium nitrite at pH values between 6.5 and 6.0, the inhibition being both more pronounced and more prolonged at the lower pH value. At pH 7.0 to 7.35 no significant inhibition of growth in the presence of sodium nitrite was observed.

In view of this result, the action of sodium nitrite on ten different micro-organisms at three pH values was tested. Acetate buffer (0.2 M) was employed to adjust the third lot of media to pH 5.7 (table II). It will be seen that, except in the case of culture 3 (a species of *Torula*), the acetate buffer tends to inhibit

growth partially or entirely in the absence of nitrite. This inhibition is probably due to the acetate as such and not to the pH, for experiments to be described have shown that acetate strongly inhibits aerobic respiration of certain of the organisms studied when employed at the same pH as phosphate buffer. However, the inhibitory effect of the acetate buffer has no direct bearing on the inhibition of growth by nitrite. It is clear from table II that growth of none of the ten organisms studied was significantly impaired by 0.02 per cent of sodium nitrite

TABLE I. Growth of two types of fish-spoilage bacteria in media of different pH in the presence and absence of 0.02% $NaNO_2$

pH of medium	No. of millions of cells per ml. of medium after the following incubation periods:					
	6 hours		24 hours		48 hours	
	Control	0.02% $NaNO_2$	Control	0.02% $NaNO_2$	Control	0.02% $NaNO_2$
*Culture 4 (*Micrococcus*). Inoculum, 9.6×10^6 cells.						
6.00	6.0	4.7	7.1	4.8	5.7	4.7
6.15	6.3	4.7	9.5	4.5	8.2	10.3
6.50	6.2	6.7	9.3	9.0	30.7	25.5
7.00	7.5	8.2	11.4	12.7	125	98
7.30	7.2	8.3	30.5	28.5	108	95
7.35	9.3	8.5	41.0	34.5	153	170
Culture 17 (*Achromobacter*). Inoculum, 4.9×10^6 cells.						
6.0	14.0	3.3	285	3.7	**	17.5
6.15	13.2	2.9	273	13.0	**	**
6.50	14.2	7.7	268	250	**	**
7.00	14.5	15.2	284	269	**	**
7.30	14.8	14.7	291	261	**	**
7.35	14.1	12.4	255	277	**	**

*Clumps counted as one organism.

**Heavy growth and pellicle formation rendered accurate counting impossible; at least 500×10^6 organisms per ml.

at pH 7.0. On the other hand, at pH 6.0, growth of all cultures, with the exception of culture 11, was impaired or totally inhibited by 0.02 per cent sodium nitrite. There was, however, a marked variation in the sensitivity of the cultures to nitrite; in certain instances the effect was transient, growth occurring after a temporary inhibition, while in other cases no growth occurred even after 14 days. Only four of the cultures grew in the medium containing acetate buffer (pH 5.7), and in all these nitrite retarded or prevented growth.

IN HALIBUT MUSCLE

Six 2 x 2 x 5-cm. fillets from a strictly fresh halibut were immersed for 2 hours at 18° C. in 1-litre portions of each of two 0.2 M phosphate buffers of pH 6.0 and 7.5. After use the pH of these solutions was 6.05 and 7.4 respectively. The fillets were drained for 10 minutes at about 18° C. on a wire screen, and

TABLE II. Growth of ten different microorganisms in media of different pH in the presence and absence of 0.02% $NaNO_2$

pH of medium	No. of millions of cells per ml. after the following incubation periods:					
	1 day		2 days		14 days	
	Control	0.02% $NaNO_2$	Control	0.02% $NaNO_2$	Control	0.02% $NaNO_2$
*Culture 2 (*Micrococcus*). Inoculum, 4.3×10^6 cells.						
7.0	10.8	10.0	32.7	27.0	+	+
6.0	1.7	0	6.3	0	+	+
5.7	0	0	0.8	0	+	+
Culture 3 (*Torula*). Inoculum, 1.4×10^6 cells.						
7.0	1.5	1.4	2.6	2.8	+	+
6.0	1.0	0	2.9	0	+	+
5.7	1.7	0	3.3	0	+	+
Culture 6 (*Flavobacterium*). Inoculum, 48×10^6 cells.						
7.0	79	64	149	159	+	+
6.0	85	0	136	0	+	0
5.7	0	0	0	0	0	0
*Culture 7 (*Micrococcus*). Inoculum, 1.8×10^6 cells.						
7.0	0.5	0.7	1.4	1.3	+	+
6.0	0	0	0.6	0.05	+	+
5.7	0	0	0.2	0	+	0
Culture 9 (*Achromobacter*). Inoculum 13.8×10^6 cells.						
7.0	154	87	400	360	+	+
6.0	37	0	320	0	+	0
5.7	0	0	0	0	0	0
Culture 11 (*Micrococcus*). Inoculum, 6.3×10^6 cells.						
7.0	3.2	3.0	37	36	+	+
6.0	0	0	1	1	+	+
5.7	0	0	0	0	0	0
Culture 16 (*Achromobacter*). Inoculum, 8×10^6 cells.						
7.0	105	68	373	284	+	+
6.0	54	0	347	0	+	0
5.7	0	0	0	0	0	0
**Culture 4 (*Pseudomonas*). Inoculum, 4.7×10^6 cells.						
7.0	504	527	1400	1320	+	+
6.0	486	82	1330	1280	+	+
5.7	0	0	0	0	+	0
**Culture C2 (*Escherichia*). Inoculum, 21×10^6 cells.						
7.0	440	410	1340	1470	+	+
6.0	384	0	1200	170	+	+
5.7	0	0	0	0	0	0
**Culture C8 (*Aerobacter*). Inoculum, 18×10^6 cells.						
7.0	540	620	1400	1520	+	+
6.0	515	35	1490	1010	+	+
5.7	0	0	0	0	0	0

*Clumps counted as one organism.

**These three cultures were originally obtained from Professor Blythe Eagles of the University of British Columbia and were not isolated from fish muscle (see Tarr 1940b).

+ Means good visible growth, normally about the same in nitrite-containing and nitrite-free media.

0 Means no visible growth by direct inspection or by microscopic observation.

then three from each pH treatment were immersed for 5 minutes at 0 to 2° C. in each of two 2-litre portions of 15 per cent by volume sodium chloride brine with and without 0.2 per cent sodium nitrite. After draining for 10 minutes at 18° C. the fillets were placed in sterile glass-covered beakers and stored at 0 to 2° C. One whole fillet was minced for each analysis, the numbers of viable bacteria, sodium nitrite content and pH being determined. It is clear from a study of table III that sodium nitrite in the concentrations present inhibited bacterial growth at pH 6.0 but not at pH 6.35.

TABLE III. Velocity of bacterial spoilage of fish muscle, adjusted to different pH values, in presence and absence of 0.0073 to 0.0085% sodium nitrite

pH of fish muscle after treatment	*Analysis of muscle after the following periods of storage					
	Immediately after treatment		5 days		12 days	
	**Bacterial count	$NaNO_2$ %	**Bacterial count	$NaNO_2$ %	**Bacterial count	$NaNO_2$ %
6.0	—	0	698×10^3	0	196×10^6	0
6.0	—	0.0073	71×10^3	0.0078	2×10^6	0.0074
6.40	—	0	1.5×10^6	0	80×10^6	0
6.35	—	0.0079	4.2×10^6	0.0081	152×10^6	0.0085

*A ground 1: 4 aqueous muscle extract as used in making bacterial counts (Tarr and Bailey 1939) was employed for all these analyses. The method followed in determination of nitrite has been described in a previous paper (Tarr and Sunderland 1940b).

**Viable count.

In another experiment six 2 x 2 x 5-cm. fillets prepared from a strictly fresh halibut were immersed for 30 minutes at 18 to 19° C. in 1-litre portions of each of the following solutions: 0.1 M $NaHCO_3-Na_2CO_3$ buffer, pH 9.5; 0.1 M citric acid−NaOH buffer, pH 6.3; 0.1 M citric acid. The fillets were drained for 10 minutes at about 18° C., and then three from each treatment were immersed for 2 minutes at 0 to 1° C. in each of two 2-litre portions of 15 per cent by volume sodium chloride brine with and without 0.5 per cent sodium nitrite. The fish was stored and examined as in the previous experiment. It is interesting to note in table IV that the amount of sodium nitrite absorbed by the fish increased with an increase in the pH of the muscle, but that the inhibitory action of the nitrite on bacterial multiplication decreased with increasing pH.

EFFECT OF NITRITE ON AEROBIC RESPIRATION

Suspensions of the organisms used were made by washing the growth from 24-hour-old nutrient agar cultures into sterile saline. No attempt was made to rid the organisms of accompanying metabolites by washing them on a centrifuge since it was desired to study the oxidation of a mixture of substrates. Barcroft differential manometers were employed to study oxygen uptake. Right-hand

cups contained the following solutions: 1 ml. of bacterial suspension; 0.45 ml. of a mixed solution of equal portions of 0.1 M glucose and sodium lactate (oxidizable substrate); 0.45 ml. of water *or* 0.12 per cent sodium nitrite solution; 0.8 ml. of 0.2 M phosphate or acetate buffers of different pH values, and 0.3 ml. of 20 per cent potassium hydroxide with filter paper in the centre cups to absorb carbon dioxide (Dixon 1934). The left-hand cups contained the same solutions, the bacterial suspension being heated for 5 minutes at 80° C., a procedure which may be conceded to destroy most respiratory catalysts present. In the case of each culture the suspension was stored at 0 to 1° C. and was used on the same day as it was prepared. Oxygen uptakes were measured after 15 minutes at

TABLE IV. Velocity of bacterial spoilage of fish muscle, adjusted to different pH values, in presence and absence of sodium nitrite (initial concentration 0.018 to 0.049%)

Analysis of muscle after the following periods of storage:								
Immediately after treatment			7 days			13 days		
Bacterial count	$NaNO_2$ %	pH	Bacterial count	$NaNO_2$ %	pH	Bacterial count	$NaNO_2$ %	pH
—	0.0180	5.50	940	0.0155	5.70	13.8×10^3	0.0105	5.80
—	0	5.35	464×10^3	0	5.65	53×10^6	0	5.95
—	0.0385	6.45	146×10^3	0.0335	6.40	8×10^6	0.0308	6.45
—	0	6.45	63×10^6	0	6.60	1660×10^6	0	7.15
—	0.0490	6.90	40×10^6	0.0360	7.00	684×10^6	0.0258	7.30
—	0	6.85	134×10^6	0	7.00	886×10^6	0	7.35

30° C. in the presence and absence of 0.02 per cent sodium nitrite at different pH. It will be observed (figs. 1 to 4) that acetate buffers inhibited respiration in all cases, just as they inhibited growth, this effect being apparently quite independent of that caused by nitrite. The influence of nitrite on the respiration of the four cultures was somewhat different in each case, and will therefore be described for each organism and compared with its effect on growth as described in previous experiments. The respiration of culture 17 (fig. 1) was not inhibited by nitrite between pH 8.15 and 6.55, but below 6.55 it was strongly retarded, in both phosphate and acetate buffers. It will be recalled that growth of this organism was also strongly inhibited by nitrite at pH 6.0. In the case of culture 2 (fig. 2) nitrite did not inhibit respiration appreciably at any pH studied, though it was previously shown that growth of this organism at pH 6.0 and at pH 5.7 was inhibited, but not prevented, by nitrite. Between pH 7.1 and 3.8 respiration of culture 9 (fig. 3) was only very slightly inhibited by nitrite, while its growth at pH 6.0 was entirely inhibited. Respiration of culture 7 between pH 6.55 and 5.2 was actually stimulated somewhat by nitrite, while its growth at pH 6.0 had been retarded, and at pH 5.7 prevented, by this salt (fig. 4). These results show that there is obviously no direct connection between the effect of nitrite on respiration and on growth.

DISCUSSION

The experiments herein described have shown for the first time that inhibition of bacterial growth by small concentrations of nitrite depends very largely on the pH of the culture medium. In many instances the action of nitrite appears to be purely bacteriostatic, inhibiting but not preventing growth entirely, while in other cases it prevents growth completely. It is possible that nitrites may act in either bacteriostatic or bactericidal capacity, depending on concentration used, length of exposure, pH of substrate, etc.; though their ability to completely inactivate (kill) bacteria is to some extent questioned by recent work in which both *Staphylococcus aureus* and *Escherichia coli* were not killed by

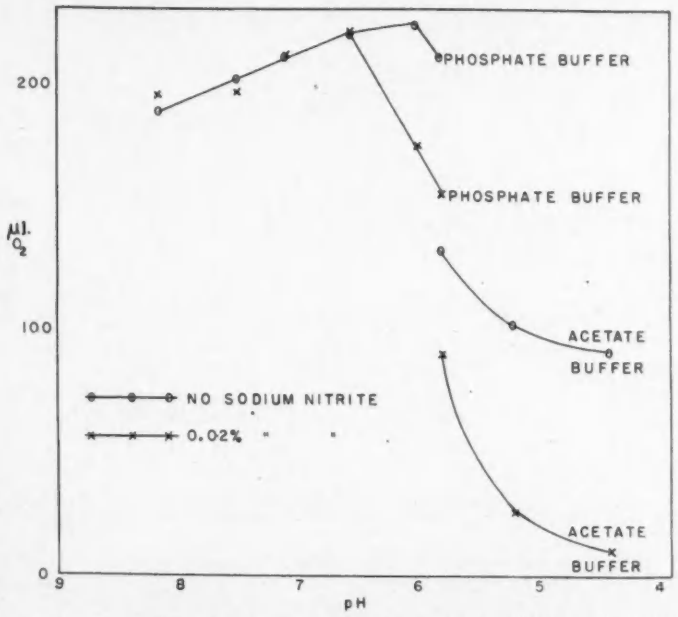

FIGURE 1. Influence of pH on the oxygen uptake of a suspension of culture 17 in presence and absence of 0.02% $NaNO_2$. 5.3 mg. (dry wt.) of bacteria in each manometer vessel.

ten minutes' contact with 38.8 per cent sodium nitrite solution at pH values between 3 and 8 (Bittenbender, Degering, Tetrault, Feasley and Gwynn, 1940). This finding is of interest in connection with curing processes in general in which nitrites are used. It indicates that, when present in brines, pickles or animal (fish) flesh in the small concentrations usually employed, they will inhibit bacterial growth, and the spoilage contingent upon such growth, only when these substances have an acid reaction.

The mechanism by which nitrites exert their toxic action is as yet obscure, though certain theories have been advanced. It is well known that sodium nitrite decomposes in acid solutions with the formation of nitrous acid, nitric acid, nitric oxide and nitrous oxide, the last-named compound combining with

water in dilute solutions to yield nitrous acid and nitric acid (Barritt 1933, Corbet 1934). The exact proportion of these compounds will undoubtedly vary with the pH of the medium, and any of them may be conceived to have a detrimental effect on the bacterial cell. It is known that sodium nitrite reacts readily with haemoglobin in the presence of a reducing agent with the formation of nitrosohaemoglobin, the reaction proceeding rapidly in acid solution (pH 5.15 to 6.63) and relatively slowly at pH 7.16 to 7.75 (Brooks 1937). The possibility that nitrite will combine with respiratory haematin compounds such as cytochrome-oxidase (Keilin and Hartree 1938) and inhibit oxidation in a manner similar to that caused by cyanide or carbon monoxide naturally occurs in view of Brook's

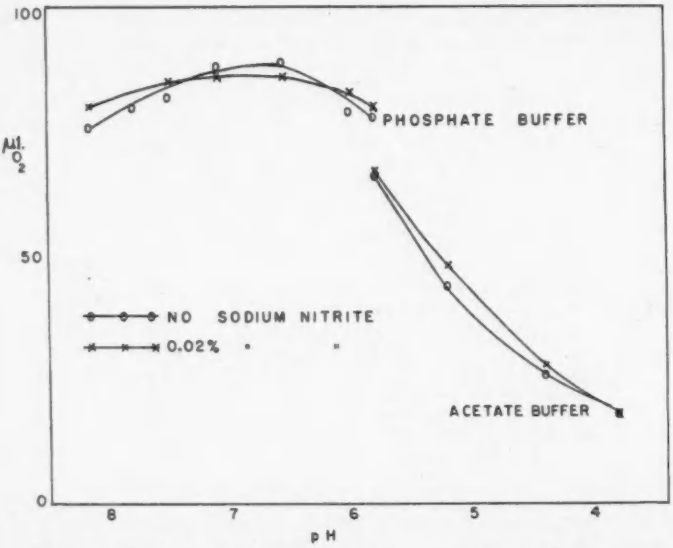

FIGURE 2. Influence of pH on the oxygen uptake of a suspension of culture 2 in presence and absence of 0.02% $NaNO_2$. 8.1 mg. (dry wt.) of bacteria in each manometer vessel.

findings. Ingram (1939) has tested this possibility using an aerobic, cytochrome-containing organism, *Bacillus cereus*, the respiration of which is strongly inhibited by cyanide. At pH 6 inhibition of oxygen uptake by this organism was complete in the presence of 0.05 per cent sodium nitrite, and very marked with 0.02 per cent of this salt. Quastel and Wooldridge (1927) found that certain of the dehydrogenase enzymes of *B. coli* were inhibited by prior treatment of suspensions of this organism in sodium nitrite solutions. Since the effect was most pronounced at acid pH, they suggested that nitrous acid was the toxic agent, and that it acted by combining with hypothetical amino groups of the enzymes concerned. As far as the facultative anaerobic types of bacteria normally associated with spoiling fish muscle are concerned, the writer's results show that it is very unlikely that nitrites inhibit growth by adversely affecting their respiration. In this connection it is of interest that the bacteriostatic action of ben-

zoates is most pronounced in an acid environment (Goshorn, Degering and Tetrault 1938). It is to be hoped that further investigation may throw more light upon the mechanism by which small concentrations of nitrite so strongly inhibit growth of certain species of bacteria.

The relation of these findings to the value of nitrites in preventing bacterial spoilage of fish is obvious, and can be summed up by stating that below pH 7.0 the more acid the reaction of the muscle the greater is the likelihood that they will enhance keeping quality. The pH of fish muscle decreases during *rigor mortis*, and varies appreciably with different species of fish (Benson 1928). Thus the pH of trawl-caught haddock muscle shortly after the onset of rigor is

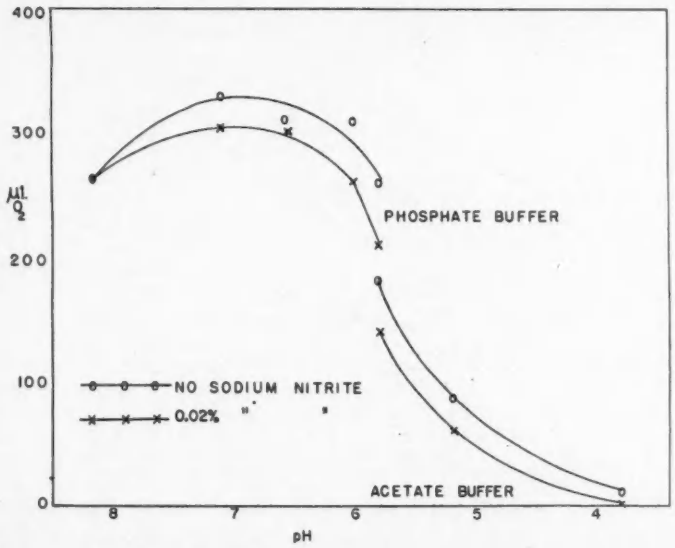

FIGURE 3. Influence of pH on the oxygen uptake of a suspension of culture 9 in presence and absence of 0.02% $NaNO_2$. 4.8 mg. (dry wt.) of bacteria in each manometer vessel.

usually about 6.3 to 6.7, and it slowly increases on storage due to bacterial production of basic compounds. Hake muscle is rather more alkaline, and during rigor the pH is normally about 7. So far no extensive study of the pH of the muscle of common species of Pacific coast fish shortly after rigor appears to have been made. The writer has observed pH values of 6.2 to 6.6 in aqueous extracts of "fresh" halibut (*Hippoglossus stenolepis*) muscle, while in similar extracts of pink salmon (*Oncorhynchus gorbuscha*) muscle pH values as low as 6.1 have been recorded. The possibility of artificially decreasing the pH of fish muscle has been considered. Experiments have shown that it is very difficult to increase its natural acidity by immersion in acid buffers or weak solutions of organic acids without either detracting seriously from its external appearance (Tarr 1940a), or subjecting it to prolonged immersion. The possibility of slightly increasing the acidity of the muscle by storing dressed fish in ice containing

small amounts of sodium acid phosphate is now being investigated, as is that of increasing the germicidal activity of ice containing sodium nitrite (Tarr and Sunderland 1940a) by the incorporation of the acid phosphate. Benzoates preserve fish quite effectively (Tarr and Sunderland 1939a, 1940b, Fellers and Harvey 1940), and it is not unlikely that their activity may also be influenced by the pH of the fish muscle. The possibility that a decrease in the pH of fish muscle, especially below about 6.0, may accelerate denaturation of proteins during freezing and storage and may also cause an increase in "drip" (Finn 1932, Reay 1933, Empy 1933, Taylor 1933, Tarr 1940a) must not be overlooked.

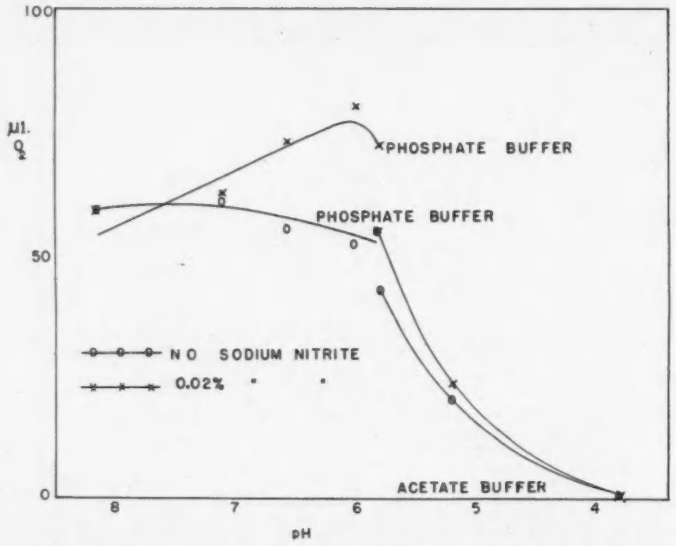

FIGURE 4. Influence of pH on the oxygen uptake of a suspension of culture 7 in presence and absence of 0.02% $NaNO_2$. 7.4 mg. (dry wt.) of bacteria in each manometer vessel.

SUMMARY

The effect of 0.02 per cent of sodium nitrite on the growth of twelve different microorganisms (four species of the genus *Micrococcus*, three of *Achromobacter* and one each of *Flavobacterium*, *Pseudomonas*, *Escherichia*, *Aerobacter* and *Torula*) in nutrient broth buffered to different pH values with phosphate or acetate buffers has been studied. At pH 7 or above nitrite did not inhibit growth significantly, while between pH 5.7 and 6.5 growth of all but two cultures (both species of *Micrococcus*) was inhibited more or less severely or completely.

Experiments with fish muscle in which the pH was experimentally altered by prolonged exposure to buffered, or weak, organic acid solutions showed that sodium nitrite only inhibited growth of the mixed natural bacterial flora when the pH was less than 7.0, preferably pH 6.5 or lower. Possible methods of artifically lowering the pH of fish muscle in order to enhance the bacteriostatic

action of nitrites, as well as the danger of promoting denaturation of proteins and increasing drip below pH 6, are discussed.

Aerobic respiration of only one of four bacteria studied was inhibited by 0.02 per cent sodium nitrite at pH 6.55 or lower, though the growth of all in broth was severely or completely inhibited. It is concluded that nitrites do not inhibit bacterial growth by sole virtue of their toxicity toward aerobic respiratory catalysts.

REFERENCES

BARRITT, N. W. *Ann. Appl. Biol.*, **20**, 165-184, 1933.

BENSON, C. C. *J. Biol. Chem.*, **78**, 583-590, 1928.

BITTENBENDER, W. A., E. F. DEGERING, P. A. TETRAULT, C. F. FEASLEY AND B. H. GWYNN. *Ind. Eng. Chem.*, **32**, 996-998, 1940.

BROOKS, J. *Proc. Roy. Soc.*, **123**B, 368-382, 1937.

CORBET, A. S. *Biochem. J.*, **28**, 1575-1582, 1934.

DIXON, M. Manometric methods. Cambridge University Press 1-122, 1934.

EMPY, W. A. *J. Soc. Chem. Ind.*, **52**, 230T-236T, 1933.

FELLERS, C. R., AND E. W. HARVEY. *Food Res.*, **5**, 1-12, 1940.

FINN, D. B. *Proc. Roy. Soc.*, **111**B, 396-411, 1932.

GOSHORN, R. H., E. F. DEGERING AND P. A. TETRAULT. *Ind. Eng. Chem.*, **30**, 646-648, 1938.

INGRAM, M. *J. Bact.*, **38**, 613-629, 1939.

KEILIN, D., AND E. F. HARTREE. *Nature*, **141**, 870, 1938.

LANDERKIN, G. B. *Food Res.*, **5**, 205-214, 1940.

LEWIS, W. L., AND J. MORAN. *Inst. Amer. Meat Packers Bull.*, **4**, 1-141, 1928.

QUASTEL, J. H., AND W. R. WOOLDRIDGE. *Biochem. J.*, **21**, 148-168, 1927.

REAY, G. A. *J. Soc. Chem. Ind.*, **52**, 265T-270T, 1933.

STEPHENSON, M. Bacterial metabolism. Longmans, Green and Co. Ltd., London. 1-391, 1939.

TANNER, F. W., AND F. L. EVANS. *Zbl. Bakt.*, Abt. II, **91**, 1-14, 1934.

TARR, H. L. A. *J. Fish. Res. Bd. Can.*, **4**, 367-377, 1939.
Fish. Res. Bd. Can. Prog. Rep. Pac., **43**, 10-13, 1940a.
J. Fish. Res. Bd. Can., **5**, 187-196, 1940b.

TARR, H. L. A., AND B. E. BAILEY. *J. Fish. Res. Bd. Can.*, **4**, 327-336, 1939.

TARR, H. L. A., AND P. A. SUNDERLAND. *Fish. Res. Bd. Can. Prog. Rep. Pac.*, **39**, 13-16, 1939a.
Ibid., **40**, 14-16, 1939b.
Ibid., **41**, 15-16, 1939c.
J. Fish. Res. Bd. Can., **5**, 36-42, 1940a.
Ibid., **5**, 148-163, 1940b.
Fish. Res. Bd. Can. Prog. Rep. Pac., **44**, 16-17, 1940c.

TAYLOR, H. F. *U.S. Patent No.* 1,920,222, 1933.

Studies on Salt Fish

V. Studies on *Sporendonema epizoum* from "Dun" Salt Fish.

By M. Frank and E. Hess
Atlantic Fisheries Experimental Station

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ABSTRACT

A morphological and physiological study of the brown halophilic mold *Sporendonema epizoum* (Corda), commonly called *Torula epizoa*, has been made. The organism is truly halophilic, optimum 10 to 15% salt. Optimum relative humidity, 75%. Growth over wide pH range. The fungistatic properties of various preservatives, particularly the fatty acids and some of their salts, were investigated. Sodium propionate was found effective and suitable for commercial control of the organism.

The term "dun fish", according to Stevenson (1899), applied some 200 years ago to a special winter cure of large codfish, containing relatively little salt, and of a characteristic brown, almost transparent appearance. It was considered of superior quality and was consumed especially during Lent, usually uncooked. Today "dun" fish refers to salt fish, before or after drying, which are covered with small brown mold colonies over part or the whole of their surface. This brown discolouration affects the sales value of the fish, although there appears to occur no breakdown of the fish flesh by the mold, and, correspondingly, no lowering of its food value.

These fish are of very common occurrence throughout the Maritime Provinces, Newfoundland and as far north as Iceland and Norway (Høye 1907). The discolouration becomes particularly evident in the fall (October) on fish that have been stored during the warm, humid summer months, as observed in our climate and also by Høye in Norway. The causative agent of this discolouration has been described by Høye (1902, 1905, 1907, 1909) and others as *Torula epizoa* Corda, and recently has been renamed by Ciferri and Redaelli (1934) as *Sporendonema epizoum* (Corda).

As well as discolouring salt fish, the organism was found by Schoop (1937) to form rust-coloured colonies on bacon, ham and sausages. Its most common habitats, apart from contaminated salt fish, are the air and dust in and around salt fish store rooms, and the salt itself which is used in the curing of the fish serves as a carrier of the spores. They can remain viable in salt for periods of one year and over (Høye 1902, 1905, 1907 and 1909, Frank and Hess 1941).

A strain of *Sporendonema epizoum* isolated by us from salt fish, and confirmed by Ciferri (personal communication), was used in the present study.

GROWTH CHARACTERISTICS

Medium

A medium consisting of glycerol-glucose-peptone and agar (Schoop 1937) and containing 13 per cent sodium chloride, was found most convenient. Omission of the peptone from the medium stops growth. Vegetative growth takes place within three days at 25°C. with rich spore formation at five to six days.

Colony Size

Colonies vary in size, depending on the conditions of growth. The vegetative mass normally varies from 0.012 mm. to 0.024 mm. after three days' growth at 25°C. With the development of the full colony there is an increase to 0.276 to 0.9 mm. Upon exposure to various chemical or physical lethal agents there is a tendency for surviving spores to develop rugose giant colonies approaching 10 mm. in diameter.

Colony Texture

Colonies show a smooth heavy nap under the best growth conditions. When adverse conditions predominate there is a tendency to granular growth and absence of coalescence. Light brown or fawn colonies grow much more smoothly than do the chocolate brown ones.

Colony Colour

The earliest vegetable stages of growth reached on artificial media are either light yellow or white, forming a sponge-like mycelium. Later with the development of spores at the end of the hyphal stems (very little branching) the colonies take on characteristic colours. Under optimal conditions the spores in mass colony development exhibit a chocolate brown colour which is easily noted against a lighter background. The pigment contained in the spores undergoes changes under unfavourable conditions. When changes in relative humidity cause water content shifts in the immediate environment of the organisms, or when the salt concentration or the temperature varies, the colouration will change through a number of stages (Frank and Hess 1941). There may be shades from whitish brown, fawn and greenish brown to dark, and chocolate brown.

Spores

Pseudo-endogenic spore formation is the main characteristic of the genus *Sporendonema* (Desmazières, emend. Oudemans, and used by Ciferri and Redaelli, 1934, to unite a large number of synonyms into one entity). This has been confirmed for the strain employed in this study. The average spore size of this strain of four to five micra corresponds with that reported in the literature.

FACTORS AFFECTING GROWTH

Salt Concentration

In Schoop medium with different concentrations of sodium chloride, at 25°C. the organism developed macroscopic brown colonies within two months' time

over a range from 5 to 26.5 per cent (fully saturated) salt with the optimum between approximately 10 and 15 per cent. It is concluded from these and similar tests that it is a true halophile.

TABLE I. Effect of salt concentration on growth rate of *Sporendonema epizoum* at 25°C. on Schoop medium

Salt added to 100 ml. medium (g.)	Days for growth to become visible		
	Unbuffered	Buffered	Buffered
0	No growth	No growth	No growth
2	No growth	No growth	No growth
5	No growth	41	20
10	7	6	3
15	5	6	4
20	7	11	21
25	9	29	..
36	35	29	..

MOISTURE AND SALT CONTENT OF UNDRIED SALT FISH

Heavily contaminated undried ("green") salt fish with a moisture content of 55 per cent and a wet weight salt content of 18.5 per cent will usually show excellent growth of brown colonies within three weeks from time of contamination, at 25°C. By varying the amount of salt and water contained in the fish muscle it is possible to cut down the time for appearance of growth to as little as four days. Høye (1909), studying hygroscopic qualities of various solar salts, found that differences in degree of hydroscopicity had no influence on the development of *Torula epizoa*.

The effects of varying salt and water content of the fish muscle upon the growth rate of *Sporendonema epizoum* may be seen from tests carried out on various undried fish, and given in table II. Knowing the water contents of the samples used, an approximate estimation of the salt concentration in the moisture of the fish muscle was made.

From these results it is evident that the "dun" condition will appear more rapidly following contamination on lightly salted fish than on heavily salted fish, but that the latter are also easily contaminated and eventually discoloured by the mold growth.

RELATIVE HUMIDITY

According to an early paper (Lesage 1895, quoted by Lutman 1929), the spores of non-halophilic molds (*Penicillium, Aspergillus*) stop germinating if the

relative humidity falls below 82 per cent. Groom and Panisset (1933) observed that *Penicillium* conidia would germinate on glass slides at relative humidities of from 81 to 100 per cent over their whole temperature range of growth (1 to 25°C.), but would cause mildewing on book materials at relative humidities as low as 72.6 per cent at room temperature. Relative humidity limits were slightly lower at 25°C. than at 10°C. and the germination rate increased with higher relative humidities.

TABLE II. Effect of moisture and salt content of undried salt fish on the growth rate of *Sporendonema epizoum* at 25°C.

Salt (% of wet weight)	Water (% of wet weight)	Salt (g. per 100 g. water)	Days for growth to appear
12.7	60.8	20.9	4
14.9	58.2	25.6	5
15.4	55.6	27.7	7
16.6	55.2	30.1	10
17.3	52.0	33.3	17
18.5	50.0	37.0	17

In order to test the effect of the degree of relative humidity on the growth of the halophilic *Sporendonema epizoum*, a series of Spray dishes were set up, containing each a relatively large amount (150 ml.) of adequately diluted sulphuric acid to obtain various relative humidities in the headspace of the sealed dishes, at 25°C. One ml. of Schoop medium each, containing 13 per cent salt, was allowed to solidify on sterile glass slides. The slides were then inoculated and suspended in the headspace of the various Spray dishes, which were sealed immediately and incubated at 25°C. Some vegetative growth only occurred at 60 per cent relative humidity and no growth was observed at lower values. At 75 per cent relative humidity growth and spore formation were at an optimum. This is in close agreement with results obtained by one of us (E. H.) on obligate halophilic red bacteria (unpublished data). Higher relative humidities, e.g. 95 per cent, such as are required for the growth of non-halophilic micro-organisms, cause a lowering of the salt concentration at the surface of the medium through hygroscopic action, and thereby retard or inhibit the growth of obligate halophiles.

TEMPERATURE

Incubation of cultures of the test organism at various temperatures on Schoop medium containing 13 per cent salt and on undried salted fish was carried out, resulting in the data given in table III. Apparently the lower limit for growth varies between 5 and 10°C. while the upper limit lies around 30°C. The zone of most rapid growth is approximately 25°C. For effective control of this organism by means of cold storage a temperature of 5°C. or lower is indicated.

Size of Inoculum

While to a certain extent the size of the inoculum determines the number of colonies arising per unit surface of medium or salt fish, it has comparatively little influence on the growth rate. Inoculation of a 9 per cent salt Schoop medium in petri dishes with a small number of spores (80) produced spore formation at 25°C. in 9 days; inoculation with 3,400 spores reduced this period to 7 days and a 340,000 spores inoculum further reduced the period to 5 days. Under less favourable circumstances—20 per cent salt—visible growth (spores) appeared after the same length of time, 9 days, with all 3 inocula, and, similarly, in a medium saturated with salt, growth became visible after 35 days for all 3 inocula. Using the

Table III. Effect of temperature on the growth rate of *Sporendonema epizoum*

Temperature (°C.)	Days for growth to appear			
	Schoop medium with			Undried salt fish, salt 18% of wet weight of fish
	13% salt	17% salt	20% salt	
5	No growth	No growth	No growth	No growth
10	17	28	30	41
15	12	17	28	22
25	5	7	9	15
37	No growth	No growth	No growth	No growth

same 3 inocula on undried salt fish—55 per cent moisture and 18.5 per cent salt, wet weight—visible growth (spores) also appeared at the same time in all three cases after 15 days.

Exposing sterile media in petri dishes to a spray of an emulsion of the spores for equal periods of a few seconds resulted in the growth of two colonies per square cm. of surface from a light spore emulsion of five spores per ml. and 300 per sq. cm. for a heavy spore emulsion of 3,000,000 per ml. Spore formation appeared after 26 days with the light emulsion spray, and was visible after 21 days with the heavy one.

Ultra-Violet Light (Sterilamp)

The commercial use of ultra-violet light in the control of mold growth in food-handling plants, such as bakeries and meat-curing stores, has recently received much attention. While Broadbent (1938) reported killing times of the order of about 25 to 35 seconds for such bacteria as *Es. coli*, *Staph. aureus* and *albus*, *B. subtilis* and yeasts, molds required considerably longer for destruction, namely four to eight minutes for *Rhizopus nigricans*, six to twelve minutes for white *Penicillium* and as high as nineteen minutes for black molds. Sharp (1939) found that approximately twice as much energy is required to kill spores of bacteria as is necessary to destroy the vegetative forms. Tomkins (1937a) noticed that on exposing meats, eggs, oranges to ultra-violet light for short periods at

regular intervals a reduction in the production of aerial mycelium of various molds occurred, causing a delay, but not checking the onset of visible mold growth.

Two different tests were made to investigate the resistance of *Sporendonema epizoum* spores to ultra-violet light. As source of light, the commercial type of Westinghouse "Sterilamp", 2537 Å units, was used. Schoop medium plates, the surface of which had been inoculated uniformly with a heavy suspension of spores, were exposed to the lamp at a distance of four inches (10 cm.), for 80 minutes. After 18 days' incubation at 25°C. an average of five colonies per plate were counted. These colonies were large and of abnormal appearance.

Droplets of a heavy suspension of spores in 0.85 per cent saline were spread on cover glasses. The dried cover glasses were exposed to the lamp at four inches distance for 0, 5, 15, 30 and 60 minutes. The dried droplets were recovered in 0.1 to 0 5 ml. saline with a Pasteur pipette and seeded on Schoop agar plants. The number of colonies developing at 25°C. was increasingly smaller with increasing exposure, but at the same time they became larger in size. Growth appeared in 7 days after 0 and 5 minutes' exposure, in 12 days after 15 minutes' exposure, and in 12 to 20 days after 30 or 60 minutes' exposure.

These results indicate that halophilic organisms have an exceptionally high resistance to ultra-violet light, which has been found by one of us (E. H., unpublished data) to hold for halophilic red bacteria of the genus *Serratia*.

Hydrogen-ion Concentration

Hjorth-Hansen (1939), using a somewhat crude technique of pouring various calculated quantities of acid or base on the freshly poured salt-wheat flour medium of Høye to obtain cultures growing at different hydrogen-ion concentrations, found *Torula epizoa* to grow and sporulate between pH 4.2 and pH 8.5. Such media have been found to exhibit great variation of hydrogen-ion concentration, particularly on alkaline plates (Frank and Hess 1941).

In order to avoid this error Schoop media, buffered effectively with McIlvaine's buffers at various hydrogen-ion concentrations, were used in our tests. Growth and spore formation occurred over a wide range, as shown in table IV. Maximum sporulation took place between pH 4.5 to 5.0. Gibbons (unpublished data) noticed that when he buffered the salt used in curing fish below pH 5.0, conditions for contamination of the fish with *Sporendonema epizoum* became very favourable.

Table IV. Effect of hydrogen-ion concentration on growth of *Sporendonema epizoum on* buffered Schoop medium (?, no growth in 21 days)

pH after autoclaving.....	2.5	3.3	4.0	5.5	7.0	7.4	8.0
Days for growth to appear.....	?	6	4	4	4	6	?

As pointed out in the next section, the effectiveness of many chemical preservatives tested to control the growth of *Sporendonema epizoum* is greatly influ-

enced by the hydrogen-ion concentration of the preservative solution. Tomkins (1937b) pointed out the same relationship in experiments on *Trichoderma*.

Chemical Preservatives in Medium

Many chemical preservatives used in the preservation of foods to cut down bacterial spoilage, have also an inhibitory effect on molds, acting as fungistatic or fungicidal agents. It has been known for some time, for example, that chemical compounds belonging to certain fatty acids, found in natural foods, have a specific fungistatic effect. Salts of one of these fatty acids—propionic acid, a natural constituent of some cheeses—are widely used in the baking industry to delay the onset of mold spoilage of bread products. The effect of a number of such chemicals in varying concentrations in Schoop medium, on the growth of *Sporendonema epizoum* was tested, with results as given in table V.

Table V. Effect of various fungistats on growth of *Sporendonema epizoum* on Schoop medium (+, growth ; −, no growth)

Fungistat	pH	Concentration of fungistat (% by weight)				Days observed
		0.1	0.2	0.3	0.4	
Propionic acid	5.0	+	−	−	−	22
Butyric acid	5.0	+	+	−	−	22
n-Caproic acid	5.0	−	−			30
n-Caprylic acid	5.0	−	−			30
n-Capric acid	5.0	−	−			30
Lauric acid	5.0	−	−			30
Calcium propionate	5.0	+	−	−	−	30
Sodium propionate	5.0	+	+	−	?	30
Sodium nitrite	5.3-6.0	+	+		+	21
Sodium benzoate	5.0			+		21

Cruess and Richert (1929) found that much more sodium benzoate was required to prevent the growth of two *Penicillium* molds, among various microorganisms, at hydrogen-ion concentrations near neutrality, e.g. pH 5 to pH 8.5, than at those in the moderately acid range, pH 2.5 to 4.5. This influence of the hydrogen-ion concentration upon the effect of chemical preservatives may also be seen from the results given in table VI.

In table VII the minimum effective molar concentrations at different hydrogen-ion concentrations of various fungistats which are necessary to inhibit growth of *Sporendonema epizoum* on Schoop medium, are given.

CONTROL

Effective control of the "dun" contamination of salt fish by *Sporendonema epizoum* must include the whole period from the preparation of the fish, storage, shipping, distribution, until it reaches the consumer. Temporary remedies merely tend to provide temporary delay of growth, and will not prevent re-contamina-

TABLE VI. Influence of hydrogen-ion concentration on effectiveness of fungistats in Schoop medium upon the growth of *Sporendonema epizoum* (+, growth ; −, no growth)

Fungistat	Concentration (% by weight)	Hydrogen-ion concentration (pH)					
		3.5	4.1	4.5	5.0	6.0	7.0
Propionic acid	0	+	+	+	+	+	+
	0.1	−	−	+	+	+	+
	0.2	−	−	−	+	+	+
	0.3	−	−	−	−	+	+
	0.4	−	−	−	−	+	+
Butyric acid	0.3			−	−	+	+
	0.4			−	−	−	+
	0.6			−	−	−	+

tion. General cleanliness and sanitation in the fish-curing plants and storehouses will help to minimize the chances of contamination and thereby reduce the losses, but they will not by themselves eradicate the "dun" condition. Hjorth-Hansen (1939) recommends the burning of sulphur to disinfect salt fish storerooms, while Høye (1907) suggested formaldehyde or sulphur.

Cold storage of the fish at 0° to 5°C. would be an ideal method of inhibiting growth, if it could be applied during the whole period from production to consumption. Since salt fish are consumed mainly in countries with sub-tropical climates in markets that are very sensitive to any increase in prices, the economic problem involved appears to exclude this method of control.

The practical ineffectiveness of ultra-violet light on the spores and the wide pH range of growth eliminate also these approaches to effective control. Furthermore, relative humidities below 70 per cent which would inhibit growth, are not feasible for the commercial storage of salt fish. There remains the possibility of

TABLE VII. Minimum molar concentrations of fungistats at different hydrogen-ion concentrations inhibiting growth of *Sporendonema epizoum* on Schoop medium

Fungistat	Hydrogen-ion concentration (pH)			
	5.0	6.0	7.0	8.0
Propionic acid	0.001	0.03	0.09	0.02
Butyric acid	0.002	0.02	0.04	0.06
n-Caproic acid	0.0005	0.001	0.005	0.005
n-Caprylic acid	0.0006	0.001	0.005	0.005
n-Capric acid	0.0006	0.0005	0.005	0.005
Calcium propionate	0.005	0.05	0.08	0.02
Sodium propionate	0.005	0.06	0.09	0.02
Calcium butyrate	0.005	0.01	0.09	0.02
Sodium butyrate	0.005	0.02	0.06	0.04

the use of preservatives effective against the spores of *Sporendonema epizoum* and suitable in the preparation of salt fish.

Preliminary laboratory tests applying various fungistats by means of "dips" of contaminated blocks of salt fish in prepared brines were carried out. Kench-cured fish, cut into squares 2″×2″ (5×5 cm.), were sprayed with *Sporendonema epizoum* spore emulsions, dipped for varying times, and incubated at 25°C. for 32 days. The minimum effective molar concentrations of the fungistats for various dips are given in table VIII.

TABLE VIII. Minimum effective molar concentration of fungistat for dips of salt fish contaminated with *Sporendonema epizoum*

Fungistat	Diluted in distilled water			Diluted in 25% salt solution		
	Length of dip			Length of dip		
	10 sec.	1 min.	5 min.	10 sec.	1 min.	5 min.
Propionic acid	0.6	0.4	0.4	0.5	0.5	0.5
Butyric acid	0.7	0.15		0.2	0.2	0.2
Caproic acid	0.05	0.02	0.02	0.05	0.05	0.04
Calcium propionate	1.0	1.0	0.2	0.2	0.2	0.2
Sodium propionate	1.0	1.0	1.0	0.4-0.8	0.4	0.4
Calcium butyrate				0.2	0.2	0.2
Sodium butyrate				0.2	0.2	0.2
Sodium caproate				0.04	0.04	0.02
Magnesium benzoate	0.2	0.2	0.05			

The use of sodium propionate was finally given a commercial test in the warehouse of a salt fish firm in Halifax. A parcel of lightly salted and one of heavily salted fish were divided into six lots each, each lot amounting to about half of one quintal (50 kg.). In making up the baths for dipping the fish, one series was prepared by dissolving the sodium propionate in fresh water, while for the other series a saturated salt brine was used as diluent. The strengths of the fungistat used in both series were 0.2 molar, 0.4 molar and 0.8 molar. Each bath had a volume of approximately 5.3 gallons (24 l.). The fish were dipped for a standard time of 30 seconds, allowed to drain and then stored in the warehouse under conditions favourable for the development of *Sporendonema epizoum* (summer weather). The height of the piles was about two feet (60 cm.) from the floor. Untreated control fish were piled alongside the treated fish.

The experiments were set up at the end of July, and the fish were examined after one and two months. First examination showed the controls free of growth, while the second examination showed positive controls. These were heavily contaminated throughout the piles of both the lightly and heavily salted fish. Growth appeared first and was heavier on the skin side of the control fish than on the cut surface and the napes.

In the treated fish (low concentrations of fungistat) growth, after two months, was restricted to the skin side of the fish. It appears that the skin offers a preferable environment to growth of *Sporendonema epizoum*, but that the contamination will eventually spread over the cut surface as well, where it is more easily visible. Whether the skin contains any specific growth stimulating substance, or whether it is merely a matter of a relatively higher moisture content has not been determined.

The results of the examination of the test lots after two months' storage show that the 0.8 molar solutions inhibited growth completely in all cases, while 0.2 and 0.4 molar concentrations were ineffective, except that in the case of heavily salted fish the use of saturated brine over water as diluent seems to increase the fungistatic effect to a certain extent. No change in these results is apparent during winter storage conditions. (Nor after a total storage of 10 months, to June, 1941).

The amount of dip solution picked up per quintal of fish was found to be approximately 3 litres (0.66 gal.). Using saturated brine as diluent some salt face develops on lightly salted fish. With heavily salted fish there appears to be no difference in the surface for the two different diluents.

SUMMARY

The characteristic brown colour of *Sporendonema epizoum* colonies is due to pigment contained in the spores. The colour varies with temperature, salt concentration and relative humidity of the environment.

The organism is truly halophilic, requiring a minimum of 5 per cent salt, the optimal salt concentration lying between 10 and 15 per cent. Optimal relative humidity for growth and spore formation lies around 75 per cent.

Growth occurs most rapidly around 25°C.; the lower temperature limits are between 5 and 10°C. and the upper around 30°C.

Ultra-violet light has comparatively little lethal effect on the organism.

The hydrogen-ion concentration range over which the organism will grow is quite wide—pH 3.3. to 7.4. Maximal spore formation occurs at pH 4.5 to 5.0.

A number of chemicals, particularly fatty acids and some of their salts, have been tested for their fungistatic effects on the organism on laboratory media and when applied as dips to contaminated salt fish. Their effectiveness is greatly increased by an increase in the hydrogen-ion concentration of the solution in the range pH 8.0 to 3.5. Propionic acid and its sodium salt have been found effective and suitable means to control the growth of the organism in laboratory tests and an experiment with sodium propionate on a commercial scale proved also successful.

REFERENCES

Broadbent, H. S. *Food Industr.*, **10**, 327, 1938.
Ciferri, R., and P. Redaelli. *J. Trop. Med. Hyg.* (1933), **36**, 167-171, 1934.
Cruess, W. V., and P. H. Richert. *J. Bact.*, **17**, 363-372, 1929.
Frank, M., and E. Hess. *J. Fish. Res. Bd. Can.*, **5**, (3), 287-292, 1941.

GROOM, P., AND T. PANISETT. *Ann. Appl. Biol.*, **20** (4), 633-660, 1933.
HJORTH-HANSEN, S. *Aarsberetn. Norg. Fisk.*, **1936** (3), 26-31, 1939.
HØYE, K. *Bergens Mus. Aarb.* **1901** (2), no. 7, 1-40, 1902.
1904 (3), " 9, 1-106, 1905.
1906 (2), " 12, 1-64, 1907.
1908 (1), " 4, 1-29, 1909.
LUTMAN, B. F. Microbiology, McGraw-Hill Book Co., N.Y., 1929.
SCHOOP, G. *Deutsche Tierarztl. Wochenschr.*, **45**, 621-624, 1937.
SHARP, D. G. *J. Bact.*, **37**, 447-460, 1939.
STEVENSON, C. H. *U.S. Fish Comm. Bull.* **1898**, 396, 1899.
TOMKINS, R. G. *Rep. Food Inv. Bd. Gr. Brit.* **1936**, 32-33, 1937a.
Trans. Brit. Mycol. Soc., **21**, 126-132, 1937*b*.

Studies on Salt Fish. VI.

Halophilic Brown Molds of the Genus *Sporendonema* emend. Ciferri et Redaelli

By M. Frank and E. Hess
Atlantic Fisheries Experimental Station

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ABSTRACT

Twenty-six cultures of brown halophilic molds from undried and dried salt fish, air and salt were compared for halophilism, temperature and pH limits for growth and ability to utilize gelatin and sugars. Nineteen cultures, including all salt fish isolations, are *Sporendonema epizoum* (Corda) Ciferri et Redaelli. They are true halophiles, show no growth at 37°C., no gelatin liquefaction; spores medium to dark brown, large. Other strains, resembling *Torula minuta* Høye, are facultatively halophilic, grow at 37°C., liquefy gelatin; spores light brown, small.

During a study of the causative agent of "dun" in salt fish (Frank and Hess 1941), a collection of 26 cultures of brown halophilic molds from various sources was obtained.

The organisms grow on a wide range of media, attesting to their widespread occurrence in nature. Very convenient for isolation purposes was Høye's (1902) wheat flour-salt paste, which interfered with the growth of bacteria and contaminating molds. The medium was especially useful in isolations from salt samples. For a comparative study of the 26 cultures a medium consisting of salt-glycerol-glucose-peptone and agar (Schoop 1937) was found most convenient. Vegetative growth took place within three days at 25°C., with rich sporulation at five to six days.

The sources of the cultures under investigation can be listed as follows: salt fish (strains nos. 1, 3, 4, 5, 6, 7, 8, 9—Halifax; no. 2—Newfoundland); solar salt (strains nos. 14, 20—unknown origin; nos. 11, 16—Inagua salt, freshly imported; nos. 15, 17, 18, 19—Turk's island salt, stored one year); air (strains nos. 10, 23, 24, 26—salt fish store; nos. 21, 22, 25—laboratory); floor dust (strain no. 13—salt fish store); dates (strain no. 12—from Ciferri and Redaelli collection).

Effect of Salt Concentration on Growth and Colour

Schoop (1937), working with two strains isolated from ham and bologna sausage, showed both to be obligate halophiles, the bologna strain requiring at

least three per cent sodium chloride for growth, the ham strain at least seven per cent.

Our 26 strains were planted on Schoop medium of varying salt content. Observations of the character of the resulting growth were made over a period of 40 days.

Seven strains (nos. 11, 12, 16, 17, 23, 24, 26) were found to be facultative halophiles, growing as well in the absence of salt as at medium high salt-concentrations, 15 to 20 per cent NaCl, and failing to grow at 26 per cent. The other 19 strains were true halophiles, five (nos. 2, 14, 18, 19, 22) requiring at least five per cent salt, the remaining 14 at least ten per cent. All grew at 26 per cent salt (saturation). All of the salt fish isolations belong to the halophilic group.

The colour of the colonies, as pointed out in a previous paper (Frank and Hess 1941) is caused by the pigmentation of the spores. It was affected to a large extent by the salt content of the medium, being darker at the low salt concentrations and lighter at the higher ones. The facultative halophiles were chocolate brown in the absence of salt, medium brown at low salt concentrations and fawn at the higher concentrations. Practically all of the halophiles exhibited also a chocolate-brown colour at their lower salt limit, a fawn colour at 15 per cent salt and mostly greenish-brown or fawn colouration at the high salt concentrations. Schoop (1937) also observed that the salt requirements and the pigmentation varied with different strains isolated by him from salt fish, ham, bacon and sausages.

Spore Size

The average size of the spores of all the salt fish strains was from four to five microns as was the size of the spores of the halophilic air and salt strains. The spores of those air and salt strains (nos. 11, 16, 17, 23, 24, 26) which were facultatively halophilic had a definitely smaller diameter of the order of 2.5 to 3 microns. Malevich (1936) reported much larger spores, having a diameter of from 6.5 to 11 microns from salt fish isolations but the average sizes of *Sporendonema epizoum* spores from miscellaneous sources are described in the literature as from three to five microns.

Effect of Temperature on Growth and Colour

The different strains were incubated on the Schoop medium at 5°, 10°, 15°, 25°, and 37°C., and their ability to grow at these temperatures was noted. The term "growth" in this paper signifies the visible appearance of brown colonies, due to spore formation, using a Zeiss binocular of 50× magnification.

As found previously (Frank and Hess 1941) no growth was observed over a period of two months at 5°C. At 10°C., only one strain (no. 11) failed to grow, while all strains grow well at 15° to 25°C.; only six strains (nos. 10, 16, 17, 23, 24, 26) grew at 37°C., five of which are facultative halophiles.

In these tests the changes in colour with temperature of incubation were also noted. All facultatively halophilic strains (nos. 11, 12, 16, 17, 23, 24, 26) exhibited a light brown (fawn) pigmentation of the spores over the whole temperature range from 10° to 37°C. The majority of the halophilic strains, including all salt

fish isolations, on the other hand, were medium brown in colour at 10°C. and chocolate brown at 15° to 25°C. Exceptions were: salt strains no. 15—medium brown over the whole temperature range, and no. 18—medium brown at 10° and 25°C. but chocolate brown at 15°C.; and air strain no. 25—lighter at 25° than at 10°C.

Effect of Hydrogen-ion Concentration on Growth

Hjorth-Hansen (1939), found that *Torula epizoa* Corda isolated from Norwegian salt fish showed growth on artificial media over a range in pH from 4.2 to 8.5. On media adjusted beyond these limits growth was one-third the rate. An attempt to repeat his work, using his modification of the Høye (1902) medium, adding codfish extract plus peptone, salt and wheat flour, failed. The adjustment of media by the method advocated by these authors, i.e. pouring acid or base directly on the surface of the medium, was found to result in serious errors. Using the Beckmann glass electrode, our work showed a spread as high as 1.6 units of pH from point to point in the medium. According to Shewan (personal communication), using the quinhydrone electrode and Hjorth-Hansen's method, there is a variation in hydrogen-ion concentration between pH 6.9 and 9.0 on the alkaline plates.

In order to avoid this error, McIlvaine's buffers were employed, the citric acid and Na_2HPO_4 having no specific effect on the mold.

For 23 strains, including all salt fish strains and halophilic isolations, there is good growth in the range pH 4.0 to 8.0 inclusive. The facultative halophilic strains nos. 12 and 26, date fruit and air isolations respectively, did not grow at pH 4.0, while strains nos. 12, 26 and 16, the latter a salt isolation, did not grow at pH 8.0. Retardation effects produced by pH changes apparently require a relatively high acidity or alkalinity. Undried salt fish extracts show hydrogen-ion concentrations, using the Beckmann glass electrode, of pH 6.0 to 6.3.

Utilization of Sugars

The Schoop medium without the addition of agar was used. Glucose, maltose, sucrose, lactose, galactose and xylose in one per cent concentration were added to separate batches. Brom-cresol-purple was used as indicator. After 35 days at 25°C., following good surface growth, the tubes were examined for acid production.

All 26 strains produced acid from glucose and xylose, but only three (nos. 9, 24 and 25) attacked lactose. Twenty-one strains, including all salt fish isolations, formed acid in maltose, galactose and sucrose. Strain no. 16 produced acid from maltose and sucrose, but failed to ferment galactose; nos. 12 and 20 attacked galactose and sucrose but not maltose; no. 17 produced acid from galactose but not from maltose or sucrose, and no. 26 fermented sucrose but not maltose or galactose. In general, the facultative halophilic strains showed more variation in their ability to ferment different carbohydrates than the halophiles did.

GELATIN LIQUEFACTION

According to the work of Ciferri and Redaelli (1934), who used a gelatin medium enriched with sugar and nutrient broth, six of their 21 strains showed signs of gelatin liquefaction after ten days' growth. Two of them, both isolated from pathological cases at Moca La Vega (Dominican Republic), showed good liquefaction, while one strain, isolated from a pathological source in Italy, showed slight liquefaction. The point of interest here would be to note the relation of parasitism to liquefaction of gelatin. The possible use of this relation as a contributory piece of evidence for potential pathogenicity of a given strain is indicated.

Malevich (1936) isolated two strains of an obligatory halophile having a lower salt limit of five per cent from salt fish. One of the strains liquefied gelatin, the other did not. Morphologically and culturally the organisms isolated by him (*Oospora nikitinskii*) appear to be similar to those isolated here.

Regular Schoop medium, substituting 15 per cent gelatin for the agar, was used. The tubes were inoculated and set up at 25°C. After 32 days the tubes were removed to the ice chest, withdrawn after a few hours and examined for liquefaction.

The results show that nine strains, including all the seven facultative halophiles and only one salt fish strain, no. 7, liquefied gelatin. It is interesting in this connection to mention Schoop's (1934) report on the isolation from the surface of salt water fish of facultative halophilic bacteria, some of which he found to be pathogenic, while none of the obligate halophilic bacteria isolated from pickled fish were pathogenic.

DISCUSSION

Ciferri and Redaelli (1934) in a comprehensive review of widely occurring brown mold organisms variously termed *Torula*, *Oospora*, *Penicillium*, *Catenularia*, *Scopulariopsis*, *Hemispora* and *Sporendonema*, group them all into the genus *Sporendonema*. Chief reasons for doing so are based on morphological and cultural grounds. The main characteristics of the genus are pseudoendogenic production of conidia, optimum growth in the range 23° to 25°C. and in media containing from one to ten per cent sodium chloride, growth in saturated sucrose solution, and brown pigmentation of spores. While not excluded as a pathological agent, the authors note their rarity as a specific cause of disease. They regard *Sporendonema epizoum* as a secondary invader in those cases of dermatomycosis where it had been isolated.

Three of our salt fish isolations (strains nos. 1, 2, 3) all belonging to the true halophilic group, were submitted to Ciferri and confirmed by him (personal communication) as typical *Sporendonema epizoum*. He also reported that Redaelli had been able to isolate such strains from samples of salt fish sold in Italy.

Høye (1907) isolated, apart from *Torula epizoa*, "the salt fish torula proper," a related species, *Torula minuta*, from the air in and around salt fish store rooms on many occasions but found it to grow only very rarely on salt fish. Its original habitat appears to be kelp. *Torula minuta* is described as growing in the absence of salt, although reluctantly, with an optimum salt concentration of ten per cent

and a maximum of 23 per cent. It resembles our facultatively halophilic group also in that the spores were found to be smaller than those of *Torula epizoa*, of the order of 2.3 to 3 microns. It is of a lighter brown colour than the spores of the latter species. In addition the colony texture is reported to be smoother than that of *Torula epizoa*, which, as pointed out in the previous paper (Frank and Hess 1941), was found to be true also for our light brown, facultative halophilic colonies in contrast to the rougher, darker brown obligate halophilic strains.

In accordance with Ciferri and Redaelli's (1934) classification, this species should more properly be called *Sporendonema minutum* (Høye), n. comb.

TABLE I. Cultural characteristics of 26 *Sporendonema* strains

Strain no.		1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 18, 19, 20, 21, 22, 25	11, 12, 16, 17, 23, 24, 26
Salt tolerance		min. 5-10 per cent max. 26 per cent TRUE HALOPHILES	min. 0 per cent max. 15-20 per cent FACULTATIVE HALOPHILES
Spores	Size	4-5 microns	2.5-3 microns
Pigment	No salt	—	chocolate brown
	Low salt	chocolate brown	medium brown
	Medium salt	light brown (fawn)	light brown (fawn)
	High salt	greenish brown	—
	10-15°C.	medium brown	fawn
	25-37°C.	medium and chocolate brown	fawn
Temperature range (°C.)		min. above 5° max. below 37°	min. above 5° max. above 37°
Hydrogen-ion range (pH)		4.0 to 8.0	4.5 to 7.5
Gelatin liquefaction		none	positive
Species		*Sporendonema epizoum*	*Sporendonema minutum*
Source		Including all isolations from salt fish.	Including all isolations from air and floor dust in salt-fish stores.

While Høye (1907) considers *Torula minuta* a distinct species, it is questionable whether the same attitude is taken by Ciferri and Redaelli. In their effort to regard the various organisms studied by them as an entity under the new name of *Sporendonema epizoum*, it appears that no differentiation betwen the two species of Høye was undertaken; in fact no reference to Høye's *Torula minuta* is made in their paper. This in spite of the fact that most cultures of the collection of Ciferri and Redaelli originated from human or other pathogenic sources and air, and that their description agrees more closely with that of our facultatively halophilic *Sporendonema minutum* group. The culture sent to us by these authors

(strain no. 12) as "typical" *Sporendonema epizoum* falls also into the same *Sporendonema minutum* group according to our studies. On the other hand, our strains nos. 1, 2 and 3 of the true halophilic group which were submitted by Ciferri for identification were confirmed by him to be also "typical" *Sporendonema epizoum*. As our correspondence with these authors has been interrupted by war conditions, we prefer, for the time being, to consider the two groups of organisms described in this paper as two distinct species.

SUMMARY

Twenty-six cultures of brown halophilic molds from salt fish, salt, air and floor dust of salt fish store houses have been studied and separated into two groups. The main differences in their characteristics are summarized in table I.

Nineteen of the strains, including all the salt fish isolations, are truly halophilic and belong to the species *Sporendonema epizoum* (Corda) Cif. et Red., while the other seven cultures closely resemble *Torula minuta* Høye, a facultative halophile. Conforming with Ciferri and Redaelli's classification this species should more properly be called *Sporendonema minutum*.

REFERENCES

CIFERRI, R., AND P. REDAELLI. *J. Trop. Med. Hyg.* (1933), **36**, 167-171, 1934.
FRANK, M., AND E. HESS. *J. Fish. Res. Bd. Can.*, **5**, (3), 276-286, 1941.
HJORTH-HANSEN, S. *Aarsberet, Norges Fisk.*, **1936** (3), 26-31, 1939.
HØYE, K. *Bergens Mus. Aarb.* **1901** No. 2, (7), 1-40, 1902.
Ibid., **1906**, No. 2, (12), 1-64, 1907.
MALEVICH, O. A. *Microbiology* (USSR), **6**, 813-817, 1936.
SCHOOP, G. *Deutsche Tierärztliche Wochenschr.*, **42**, 816-819, 1934.
Ibid., **45**, 621-624, 1937.

The Consumption of Young Sockeye Salmon by Predaceous Fish*

By W. E. Ricker
Pacific Biological Station

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ABSTRACT

Among the fish and insects which constitute the principal foods of the larger predaceous fish of Cultus lake, B.C., young sockeye salmon (*Oncorhynchus nerka*) are of outstanding importance. In general, the consumption of sockeye is proportional to their abundance, though it may be in some cases modified in the direction of less than proportional predation upon the smaller populations. Consumption of alternative foods increases greatly in years when sockeye are scarce.

INTRODUCTION

Following discovery by Foerster (1929) of the high rate of natural mortality among fingerling sockeye salmon in Cultus lake, an effort has been made to determine the agents responsible, together with some measure of the quantitative effect of each. Beginning 1932, gill nets were used in the lake to take samples of the fish population. Examination of the stomach contents of fish so taken revealed so large a destruction of young salmon (Ricker 1933) that it was decided to begin at the first opportunity an experiment in sockeye conservation by removing as many as possible of the predators. This experiment was begun in May of 1935, and provided abundant additional material for the study of food of the predators. The numbers of fish of each kind, taken from the lake from 1932 to 1938, have been recorded elsewhere (Foerster and Ricker 1941). The majority of these have had their stomachs examined, and a few additional stomachs have been obtained from anglers. The results presented herein have accrued from the study of this material.

PROCEDURE

From 1932 to 1934, gill-nets were handled in rather uniform fashion. A gang of about 10 nets was set from shore outward, and left in the water for periods of about 24 hours (overnight), rarely for two nights. Longer sets were not made in order to avoid loss of stomach contents through digestion while the fish remained in the net. With more intensive netting begun in 1935, it was no longer possible to make such regular visits, and nets were commonly left for two or

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three days without being "run", and sometimes for longer periods. In comparing amounts of food consumed in different years, it is necessary to segregate the period 1932-34 from 1935-38. This is desirable for another reason: starting in 1935, many nets were set singly near shore, and fish taken therein were held at a much higher temperature (except in winter) than those on the bottom offshore, and could presumably digest more of their stomach contents per unit time.

Examination of the stomachs was from the fresh fish, with the exception of those taken August, 1935 to April, 1936, which were from fish preserved in formalin. The examination of the fish was made by the writer personally from 1932 to June, 1936, assisted during times of large catches by Drs. R. E. Foerster and A. L. Pritchard. From the latter half of 1936 onward, collection of the stomach contents was done for the most part by the net operators, the whole stomach or the contents alone being placed in bottles with formalin, for later study by the writer. It is felt that small amounts of food, or perhaps materials not easily recognizable as food, may sometimes have escaped these men, but that no large quantity has been overlooked.

In the laboratory examination, the total volume of contents of each stomach separately was found by water displacement, as was that of individual items of any size. Where numerous small items were mixed together, the approximate volumetric percentage of each was estimated from inspection. No attempt was made at an exhaustive identification of the smaller food items (chiefly insects), but all fish remains were carefully scrutinized. As the number of species occurring in the lake is not great, it was possible to make an accurate identification in the great majority of cases, even of well-digested remains, by means of such structures as jaws, gill arches, spines, pharyngeal teeth, and so on. Even the stomach, which is about the last part of some fish to disappear, was rather distinctive in some cases.

No attention was given to the contents of the intestines of any fish. Any items found in the mouth were included as stomach contents. Such occurred not infrequently, and can be ascribed partly to voluntary regurgitation but more usually to pressure applied in removing the fish from the net. The question of to what extent various fish regurgitate when caught, deserves more consideration than it has had. Our impression is that Cultus fish of all kinds lose little food when caught in gill nets, and then only when they are excited as the net is drawn to the surface; but that line-caught trout often regurgitate freely, particularly if their food is of items of small individual size.

Species of Fish in Lake

A complete list of fish known to inhabit Cultus lake is as follows.

Coregonidae: *Prosopium williamsonii*. The Rocky Mountain whitefish is not a common fish, judging by its scarcity in the net catches.

Salmonidae: *Salmo clarkii*. Cutthroat trout are common.

S. gairdnerii, steelhead trout. At various times fry of this species have been placed in the lake. They were not recognized in this investigation, however, until 1937, when a few small ones were taken, apparently products

of a planting of fry in the outlet creek in 1936. Some of these may have found their way into the trout catches, and hence stomach analyses, of 1938.

Salvelinus malma, Dolly Varden char. A fairly common fish, living in the deeper waters.

Oncorhynchus kisutch. The coho salmon are common in some years.

O. nerka. Sockeye salmon normally remain in the lake one or occasionally two years, then migrate seaward. A relatively small number do not migrate, and mature in the lake. A small population of kokanee is also present (Ricker 1938).

Catostomidae: *Catostomus macrocheilus*. The sucker is common.

Cyprinidae: *Ptychocheilus oregonensis*. The squawfish, a large predatory minnow, is abundant.

Richardsonius balteatus. Shiners are an abundant small species.

Mylocheilus caurinus. The chub appears to be rare.

Ameiuridae: *Ameiurus nebulosus*. The catfish is the only non-native species, and may not be permanently established. Only one specimen was netted, and another found dead.

Gasterosteidae: *Gasterosteus aculeatus*. Sticklebacks are an abundant small fish.

Cottidae: *Cottus asper*. The prickly sculpin is abundant near shore, only a few large specimens having been taken from deep water.

C. aleuticus. The Aleutian sculpin of Cultus lake is a small species of the deep water, known only from the specimens found abundantly in char stomachs.

General Significance of Data

It will readily be conceded that fish caught in one part of a lake are not necessarily representative of the whole population. In the present instance, fish caught in bottom-set gill nets may reasonably be supposed to have more of bottom organisms and bottom-feeding fish in their stomachs, than would the general population, provided any significant correlation exists between situation and food taken. While no exhaustive study of this phenomenon could be made, the following specific points seem established:

(1) No significant differences appear in the food of squawfish or trout taken in bottom nets set inshore, as compared with floating nets in the same location.

(2) Squawfish taken in deep sets may consume more sockeye *in summer*, than squawfish taken in shore sets. As very few squawfish were taken in deep water in summer, this is not an important difference, i.e. the shallow-caught stomachs represent the great bulk of the squawfish population. Of the summer squawfish analyses tabulated, only those from 1932 to 1934, and August of 1935, consist largely of deep-caught fish. In winter little difference appears between deep and shallow catches.

(3) Trout caught in inshore nets differ in regard to stomach contents from those taken by anglers trolling offshore, at least in spring and summer. The troll-caught fish contain more plankton and possibly fewer insects (except midge pupae) than the net-caught fish—at any rate in 1934, the only year any number

of the former were examined. It would appear from their frequency in anglers' catches that a considerable body of trout inhabits the pelagic region of the lake; hence the tabulated analyses of trout stomachs include too little plankton (except perhaps in 1934) to be representative of the lake's population as a whole. Similar considerations probably apply to coho salmon.

(4) The char examined, taken almost entirely on the bottom in deep to moderately deep water, may have a larger proportion of bottom fish (*Cottus aleuticus*, *C. asper*, suckers, etc.) and a smaller proportion of pelagic fish (sockeye salmon and to a lesser extent sticklebacks) than is representative of the lake's total population. There is, however, no way to test this possibility from the data at hand.

Except where changes in fishing method have taken place, our data give comparable conditions from year to year. Changes in percentage consumption of various items can be traced, therefore, even though no assurance is available that an exact representative picture of rate of predation for the whole population is presented. Wherever changes in fishing method have occurred, care is taken to consider their possible effects when different years are compared.

Another aspect of the representativeness of these data concerns the scarcity of fish taken at times of the spawning of adult sockeye, i.e. mid-October to late December. This has resulted from two causes: (1) In 1932, 1933, 1934 and 1936 no adult sockeye were allowed into the lake, because they were retained for fish-cultural operations at the outlet. Thus in these years no opportunity was presented for a study of the normal consumption of salmon eggs and salmon offal by the fish in the lake, though it was possible to set nets and determine their food in the absence of spawning salmon. (2) In the remaining years of the study, the very presence of salmon in the lake proved an obstacle to obtaining other fish from it. Salmon very readily become entangled in nets set in the lake, both near and remote from the actual spawning grounds. Because of damage to the fish and the nets, very little netting was done while the salmon were in the lake, i.e. from about mid-October to the end of the year. In the autumn of 1935, and to a less extent in 1937, some fish were taken from the salmon spawning beds by means of traps and bait lines; these, however, rarely caught anything but squawfish and prickly sculpins. , What change the presence of the salmon in the lake effects in the food of the various fish must largely be conjectured.

Presentation of Data

Several systems of presenting the results of stomach examinations are in vogue, each with some advantages. The *frequency of occurrence* of different types of food in different stomachs, or the *average number* of individual items per stomach, can be calculated on the basis either of the total stomachs examined, or the number that contained food. The *volumetric or gravimetric percentage* of the total food present which a particular kind of food represents, may also be utilized. All three of these methods are used to some extent in the tables to follow, though most emphasis is placed on volumetric percentage and frequency of occurrence.

As this paper primarily concerns the sockeye consumed in Cultus lake, no complete exposition of the stomach contents of the various fish taken is attempted.

In tables I to IV, the frequency of occurrence and percentage by volume of the sockeye found are tabulated, along with the volume percentage of other fish (lampreys, sticklebacks, shiners, prickly and Aleutian sculpins, and others less important) and of foods other than fish (mostly insects, but including some Entomostraca, gastropods, etc.).

Tabulations of sockeye eaten are made after grouping the predators into classes of 100 mm. length intervals (to the fork of the tail), in all cases except char, where 200 mm. groups are used. Choice of these limits was largely a matter of convenience in tabulating, though the result is probably as useful a picture as would have been obtained in any other way. Except in the case of the coho, segregation by year classes would have been impossible without extensive scale reading, and would have given overlapping length groups in any case.

RESULTS

As it has proved impossible to include in this paper the whole of the available data on sockeye consumption, typical years' records have been chosen to indicate seasonal trends and differences due to the size of the predator. As indicated above, two phases of the study may be separated, that of 1932 to 1934 and that of 1935 to 1938, on the basis of a change in technique and intensity of fishing. Squawfish and char were caught in fairly good numbers in both periods, so data for 1932-33 and 1936-37 have been selected for presentation (tables I and II). Trout and coho, however, were taken in only insignificant numbers in the first phase of the study, so records of their consumption of sockeye are presented for 1936-37 and 1937-38 (tables III and IV).

Instead of the calendar year, a "sockeye year" is chosen as the time unit in each case; that is, the time from the emergence of sockeye fry from the gravel of the redds in May, to the departure of the majority of them in the following April. Some year-old fish were, however, taken in May, and a few at other seasons.

Squawfish

Young sockeye up to a year of age are the most important food of squawfish more than 100 mm. long (table I), except from May to September. During this summer period very few sockeye are found in the stomachs, principally because very few squawfish can be caught at that season in the pelagic region of the lake which the sockeye frequent. Squawfish taken inshore at this season eat coarse fish (chiefly shiners and sticklebacks), terrestrial insects, and some plankton.

In number of sockeye consumed per individual fish, squawfish stand much below the various salmonid predators. Except for occasional groups of up to 20 fry found in stomachs in May, the usual number found in a stomach did not exceed three, while the average for any large series was never greatly in excess of one, commonly only 0.2 to 0.5 per stomach.

In evaluating the absolute effect of the whole population of squawfish upon the lake's sockeye, consideration should be given to the fact that they were taken several times as abundantly in the nets as was any other predator (Foerster and Ricker 1941). Against this must be set their low rate of consumption of sockeye,

TABLE I. Sockeye and other foods consumed by squawfish, 1932-33 and 1936-37

	1932-33							1936-37						
	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish
Squawfish 100-199 mm. long														
May	3	.29	3	1	79.5	8.0	12.5	?	?	3	2	60.0	0	40.0
June								?	?	1	0	0	100.0	0
July								61	.07	6	1	19.5	39.0	41.5
Sept.	1	.50	1	0	0	100.0	0							
Nov.	21	.12	11	6	64.7	17.5	17.8							
Jan.	3	3.60	3	3	100.0	0	0							
March	4	.80	2	2	100.0	0	0							
April	5	.40	2	2	100.0	0	0							
Squawfish 200-299 mm. long														
May	3	.33	2	1	10.0	90.0	0	206	.36	63	4	4.6*	10.0	85.4
June								974	.02	115	4	6.4*	55.0	38.6
July								170	.15	22	0	0	87.6	12.4
Aug.								7	.07	2	0	0	100.0	0
Sept.								72	.27	24	12	65.6	28.2	6.2
Oct.	24	.12	7	7	100.0	0	0	267	.50	51	38	89.1	10.4	0.5
Nov.	78	.05	23	21	94.6	5.4	0	296	.36	39	37	98.2	1.8	0
Dec.								73	1.16	20	18	91.0		
Jan.	16	.78	10	10	100.0	0	0	36	.97	14	14	100.0	0	0
Feb.	5	.50	2	2	100.0	0	0	10	.97	3	3	100.0	0	0
March	26	.53	16	15	92.7	6.5	0.8	32	2.16	17	16	95.7	4.3	0
April	16	.36	4	3	91.4	8.6	0	28	.76	6	6	100.0	0	0
Squawfish 300-399 mm. long														
May	1	6.20	1	0	0	100.0	0	33	.89	13	2	6.2	14.1	79.7
June								282	.19	48	2	1.1	88.3	10.6
July								50	.45	5	0	0	80.8	19.2
Sept.								4	.38	2	1	60.0	40.0	0
Oct.	11	.65	5	5	100.0	0	0	61	1.01	9	6	58.0	41.8	0.2
Nov.	15	.03	1	1	100.0	0	0	43	.65	6	5	89.2	10.8	0
Jan.-Feb.	3	.03	1	1	100.0	0	0	6	1.75	3	3	100.0	0	0
March-April	5	.50	2	2	100.0	0	0	14	1.23	3	2	44.8	55.2	0
Squawfish more than 400 mm. long														
May								?	?	2	0	0	91.2	8.8
June								?	?	1	0	0	100.0	0

*Some of the sockeye consumed in these months were more than a year old. In all other cases, only fingerling fish were represented.

as measured by the numbers occurring in their stomachs, and the fact that most of it is during cold weather, when digestion is presumably slow. Hence although squawfish must be reckoned one of the more important consumers of sockeye it

TABLE II. Sockeye and other foods consumed by char, 1932-33 and 1936-37

	1932–33							1936–37						
	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish
Char 100-299 mm. long														
May	2	3.4	2	2	100.0	0	0	25	1.1	11	9	85.4	14.6	0
June								13	2.1	13	11	87.7	11.9	0.4
July								9	3.1	7	6	64.0	36.0	0
August								5	3.6	5	2	28.5	71.5	0
Sept								5	4.3	4	3	98.6	0	1.4
Oct								5	0.3	1	1	100.0	0	0
Nov								6	1.2	2	1	66.7	33.3	0
Dec								10	4.4	8	5	90.1	9.9	0
Jan								2	5.6	1	1	100.0	0	0
Feb								2	1.3	1	1	100.0	0	0
March								9	4.0	6	1	44.6	55.4	0
April								4	2.1	2	1	36.5	63.5	0
Char 300-499 mm. long														
May	2	4.2	1	1	100.0	0	0	44	2.3	12	4	40.2	59.8	0
June	3	12.3	3	1	3.3	96.7	0	23	5.8	18	5	31.3*	68.3	0.4
July	12	9.3	11	9	46.5	53.5	0	4	41.4	4	1	2.2*	97.8	0
August	9	7.1	8	8	99.3	0.7	0	1	1.5	1	1	100.0	0	0
Sept								6	0.3	1	1	100.0	0	0
Oct	7	2.8	3	3	100.0	0	0	30	3.0	11	9	64.9	35.1	0
Nov	7	9.2	6	6	100.0	0	0	18	3.7	8	7	45.9	54.1	0
Dec								2	14.4	2	2	100.0	0	0
Jan	2	11.2	2	2	100.0	0	0							
Feb	1	0.1	1	1	100.0	0	0							
March–April	3	8.7	3	2	78.1	21.9	0	1	5.2	1	1	100.0	0	0
Char 500 mm. and longer														
May	1	0.0	1	0	0	0	+	5	8.1	2	1	13.1	86.9	0
June	6	4.7	6	1	3.5	93.9	2.6	1	11.0	1	0	0	100.0	0
July	4	9.6	4	4	56.3	40.0	3.7							
August								1	0.5	1	0	0	100.0	0
Sept	2	3.5	2	2	100.0*	0	0							
Oct	3	2.3	1	1	100.0	0	0							
Nov	2	6.0	2	2	100.0	0	0							
March	2	5.2	1	1	100.0	0	0							
April	2	2.0	2	2	100.0	0	0							

*Some of the sockeye consumed in these months were more than a year old. In all other cases, only fingerling fish were represented.

is doubtful if their total activity exceeded that of the trout, even prior to 1935. The number of squawfish greater than 200 mm. long present in the lake in June, 1935, was very approximately estimated as 8,400 (Foerster and Ricker 1938). The present (1938) level of abundance is only about one-tenth of that. To these estimates must be added, however, a considerable number in the 100 to 199 millimetre group, which also consume sockeye.

Char

The char (table II) less than 300 mm. long have a rather uniform diet of fingerling sockeye, and of other fish, largely *Cottus aleuticus*. Char more than 300 mm. long, while keeping to these as staples, add more variety in the way of shiners, sticklebacks, squawfish and prickly sculpins. They appear to take about as many of the smallest sockeye, in May and June, as do trout. The number found in individual stomachs ranges up to 93, with 10 or 12 per stomach examined a common average. During the summer and for the remainder of the year, the char eat fewer sockeye than do trout, on the average. This may doubtless be explained by the common observation that the char is for the most part a bottom-frequenting fish, whereas the sockeye appear to leave the bottom and take up a pelagic life a short time after they emerge from the gravel of the redds.

Char succumbed so early and so nearly completely to the netting operations, that it was calculated their numbers in 1932 could not have exceeded 500 of all sizes commonly taken by gill nets, and was probably less (Foerster and Ricker 1938). At present (1938) they are much reduced in numbers, and no longer an important factor in sockeye predation, unless the smallest specimens (less than 200 mm. long) take an unusually large number.

Trout

Trout (table III) are not an almost exclusively fish-eating species like char, but take large quantities of insects as well. In general the smaller trout eat more insects, the larger more fish; though when midge pupae or ants are very abundant, almost all individuals gorge on them. Among fish, the very young larvae of *Cottus asper* are eaten principally by smaller trout. Sticklebacks are infrequent in small trout, and probably the most important food of the largest. Sockeye salmon seem most common in the intermediate size range 300 to 399 millimetres, though little less so in the 200 to 299 group.

Unlike squawfish, trout are important consumers of sockeye at all times of year. The numbers found in individual stomachs is greatest in May and early June when the fry have only recently emerged from the gravel—over 90 having been found on two occasions, while the average may be 10 to 15, or 5 to 7 per stomach examined. The number taken by the 300 to 399 mm. group, in years when sockeye are abundant, tends to decrease through the summer, after which it remains fairly steady at 2 or 3 per stomach in which they occur, or 1 to 2 per stomach examined, up to the March following. There is a small but probably significant increase in April, when the fingerlings migrate shoreward preparatory to leaving the lake. In terms of total number consumed, there is doubtless a

TABLE III. Sockeye and other foods consumed by trout, 1936-37 and 1937-38

	1936–37							1937–38						
	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of food other than fish
Trout 200-299 mm. long														
May. ..	14	3.2	10	2	3.4	29.9	66.7	16	1.8	13	4	15.7	13.9	70.4
June....	31	1.6	18	3*	19.0*	35.6	45.4	21	1.5	8	1	14.7	27.1	58.2
July....	9	1.2	5	1	56.9	27.5	15.6	10	1.5	3	0	0	94.0	6.0
August..	5	2.2	5	5	96.3	0	3.7							
Sept....	32	1.5	16	5	45.1	15.4	39.5	56	1.6	26	3	6.0	16.8	77.2
Oct.....	22	1.9	9	7	90.4	0	9.6	71	1.6	27	3	12.8	9.4	77.8
Nov....	22	3.1	13	11	95.0	2.8	2.2	13	0.5	1	1	93.6	0	6.4
Dec.....	29	2.2	16	11	83.3	14.3	2.4	97	0.4	15	13	93.8	4.1	2.1
Jan.....	10	0.9	3	2	79.8	0	20.2	20	0.9	5	4	86.3	13.7	0
Feb.....	16	0.9	2	2	55.3	34.1	10.6	50	0.6	16	2	23.5	4.4	72.1
March..	7	1.9	3	2	57.7	6.2	36.1	41	0.6	8	2	45.0	0	55.0
April....	5	1.5	4	2	53.3	12.1	34.6	48	0.9	9	2	48.5	0	51.5
Trout 300-399 mm. long														
May....	30	5.2	13	3*	4.2*	9.7	86.1	28	1.2	8	3	57.7	12.6	29.7
June....	42	2.8	31	10	23.8	65.0	11.2	20	2.2	6	1*	13.6*	34.9	51.5
July....	10	4.3	8	6	80.9	21.1	0							
Aug.....	9	5.5	9	9	98.4	1.6	0	3	2.5	1	1	100.0	0	0
Sept.....	27	3.9	23	17	81.5	14.9	3.6	8	2.2	5	2	19.5	77.6	2.9
Oct.....	41	6.3	29	26	93.1	6.4	0.5	9	4.5	4	2	46.0	48.3	5.7
Nov.....	46	4.1	31	31*	99.4*	0.1	0.3							
Dec.....	38	3.1	20	20	100.0	0	0	89	1.2	24	22	86.9	6.2	6.9
Jan.....	12	3.4	6	6	100.0	0	0	35	2.1	16	15	94.1	0	5.9
Feb.....	7	8.1	7	7	100.0	0	0	65	2.3	25	21	92.1	3.7	4.2
March..	36	5.9	27	27	99.1	0	0.9	52	3.0	18	17	90.3	0	9.7
April....	41	4.7	22	20	91.6	0	8.4	47	6.4	31	29	90.7	7.0	2.3
Trout 400 mm. long and up														
May....	12	15.8	6	2*	1.7	22.1	76.2	9	0.7	1	0	0	100.0	0
June....	15	7.6	12	2	3.7	95.9	0.4	8	1.4	2	0	0	100.0	0
August..	1		1	1	100.0	0	0	7	12.4	6	1	4.1	95.9	0
Sept.....	3	3.5	2	1	97.2	2.8	0	6	9.3	3	0	0	100.0	0
Oct.....	17	8.3	13	9	71.0	29.0	0							
Nov.....	18	2.5	8	8	95.0	5.0	0							
Dec.....	13	8.8	7	7	100.0	0	0							
Jan.....								7	1.3	1	0	0	100.0	0
Feb.....	9	1.6	3	3	100.0	0	0	15	0.6	1	1	100.0	0	0
March..	21	4.7	7	7	99.7	0	0.3	26	0.4	1	1	90.0	10.0	0
April....	36	0.9	7	6	95.2	4.8	0	61	3.5	11	11*	100.0*	0	0

*Some of the sockeye consumed in these months were more than a year old. In all other cases, only fingerling fish were represented.

decline from autumn to winter, and pronounced increase from February to April, owing to the speeding up of digestion with rising temperature.

No good estimate of the total consumption of sockeye by trout can be made, but the latter are one of the most important, and at the moment probably *the* most important predator in the lake. Whether they were in 1935 only one-fifth as common as squawfish, as the net catches suggest, is a matter of conjecture. At present they have increased greatly, relative to squawfish, though perhaps only holding their own or showing some decline in absolute numbers (Foerster and Ricker 1941).

TABLE IV. Sockeye and other foods consumed by coho salmon, 1936-37 and 1938-39

	1936–37							1937–38						
	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of foods other than fish	Number of stomachs examined	Average volume of food in ml.	Number of stomachs containing food	Number of stomachs containing sockeye	Volume percentage of sockeye	Volume percentage of other fish	Volume percentage of food other than fish
Coho 200-299 mm. long														
May	11	0.2	1	0	0	0	100.0	3	2.1	1	0	0	100.0	0
June	2	2.4	1	1	100.0	0	0							
Aug.								8	2.4	2	2	100.0	0	0
Sept.	4	0.1	2	1	75.0	25.0	0	74	2.6	40	33	88.8	11.2	0
Oct.	6	1.4	1	1	100.0	0	0	98	2.4	46	34	73.4	11.2	15.4
Nov.	20	3.2	9	9	88.5	11.5	0	2	6.8	2	2	99.2	0	0.8
Dec.	11	3.2	5	5	100.0	0	0	42	2.7	16	15	97.3	3.7	0
Jan.	3	1.5	1	1	100.0	0	0	8	0.8	4	3	80.3	0	19.7
Feb.								8	2.7	5	4	81.7	18.3	0
April								3	2.8	2	2	62.4	29.4	8.2
Coho 300-399 mm. long														
May								2	3.2	1	0	0	100.0	0
June	9	1.9	5	5	79.5	0	20.5							
Sept	2	15.7	1	1	100.0*	0	0							
Oct.	4	2.7	1	1	100.0	0	0	5	4.1	3	3	100.0	0	0
Nov.	5	1.0	2	2	100.0	0	0	1	11.0	1	1	100.0	0	0
Dec.	3	0.8	1	1	100.0	0	0	16	1.6	5	5	100.0	0	0
Jan.	2	8.2	1	1	100.0	0	0	24	4.0	12	12	100.0	0	0
Feb.								32	4.0	16	16	100.0	0	0
Mar.								26	3.0	10	10	99.9	0	0.1
April	3	5.4	2	2	100.0	0	0	45	5.0	25	24	97.6	0	2.4

*Some of the sockeye consumed in these months were more than a year old. In all other cases, only fingerling fish were represented.

COHO SALMON

From the data at hand, table IV, coho appear to be even more consistent consumers of young sockeye than are the trout, in the sense that other fish and

insects are relatively less frequent. The scarcity of sticklebacks and absence of prickly sculpins indicates that coho typically feed in the pelagic region of the lake. The average number of sockeye found per stomach examined is somewhat more than in trout of the 200 to 299 mm. range, and somewhat less in the 300 to 399 mm. range. Seasonal variation in number taken follows much the same course as with trout.

Coho have been a very variable predaceous influence. The year-class from the spawning of 1932-33 (active against sockeye from the autumn of 1934 to autumn of 1935) was fairly numerous, as were those of 1933-34 and 1935-36. From the spawning of 1934-35 not many coho were recovered in the lake, giving the sockeye a corresponding freedom from predation from autumn of 1936 to autumn of 1937. The adult anadromous coho of the spawning of 1937-38 were kept out of the lake, so the only examples of that year-class should be the few resulting from the spawning of freshwater stock.

In their years of abundance, the number of coho caught does not fall greatly short of that of trout, and may even equal the latter in a few months (March, 1936 and September and October, 1937). In such years they evidently have an important influence upon the production of sockeye migrants.

Prickly Sculpin

Being a bottom-dwelling fish found principally close to the shore of the lake, sculpins have opportunity to attack sockeye at only two rather short intervals of their lake life, namely when the fry first emerge from the redds, and when at one year of age they migrate shoreward preparatory to leaving the lake for the ocean. On both occasions a considerable toll may be taken, but since of 567 stomachs examined, only 61 contained any food, our data are too scanty to afford much of a quantitative estimate. A number of specimens taken in May, 1937, contained a large number of sockeye newly distributed from the hatchery (111 in one stomach), but these were all from one point in the lake and give little idea of average conditions. Foerster (1925) records the capture of hatchery-reared sockeye fry by sculpins in the lake.

Consumption of year-old sockeye by sculpins has been commonly observed in the creek leaving the lake, but not in the lake itself. That the larger sculpins can easily swallow fish of that size is shown by cases of cannibalism in which a sculpin more than half the length of the cannibal may be eaten.

Rocky Mountain Whitefish

The *Prosopium* taken in the lake amount to 53 specimens, but only 5 of these were noticed to have food in the stomachs; and of these, one 414 mm. long, taken June 3, 1932, contained 10 small sockeye.

Residual Sockeye Salmon and Kokanee

The food of sockeye salmon in the lake has been presented earlier (Ricker 1937). A few residual or lake-type sockeye were found to consume young fingerlings of their kind, but not in great numbers. Kokanee may do the same, but are very scarce in the lake.

PREDATION AND ABUNDANCE OF SOCKEYE

Since Cultus lake was used to estimate survival of young sockeye throughout the period of these food studies, it is possible to make some comparison of rate of predation with the actual number of sockeye present. From data of Foerster (1936, 1938 and MS.) the number of migrants leaving the lake each year may be written down, and also some estimate of the number of young sockeye which began life in that year. The sockeye eggs are spawned in autumn, hatch in early spring, and the fry become available in May when they leave the redds (or are distributed from the hatchery). They remain at large in the lake until the time of their migration seaward in the spring (chiefly April and May) of the year following.

In table V, the column "Total migrants" provides a basis of comparison for any predation occurring in late winter and early spring; it includes the yearlings and two-year-olds migrating in any given year. The column "Estimated number of fry produced" can be used for comparing with summer predation, but it is, of course, much less accurate than the migrant count.

The number of fry of each spawning to survive a year or more in the lake is indicated in the last column of the table. The sudden increase in average survival rate from 5 to 15 per cent approximately, which is noticeable in comparing fingerlings spawned in 1933 and earlier with those spawned in 1934 and later, has been attributed to removal of fish from the lake (Foerster and Ricker 1938, 1941).

TABLE V. Estimates of sockeye present in the lake as free-swimming fry (May), and the count of migrants leaving the lake (following March to May), from the data of Foerster (1938 and MS.). Free-swimming fry are estimated as follows: (1) all of the fry distributed from hatcheries, (2) 95 per cent of eyed-eggs planted (excluding 2,012,000 of the 1934 spawning which were killed by drought), (3) 50 per cent of eggs in females spawning naturally. Estimate (2) is based on the survival from experimentally-planted eggs (see Foerster 1935, and Robertson 1937 for experiments); estimate (3) is very uncertain, but is based on an assumption of similar survival rates from the free-swimming stage onward, in years of natural and artificial propagation. All figures tabulated represent thousands.

Year of spawning	Period of availability of fry and fingerlings	Eggs in females spawning naturally	Eyed eggs planted	Fry distributed	Estimated no. of fry produced	Total migrants	Total survivors of fry opposite
1930	1931–32	24,900	..	..	12,400	789	789
1931	1932–33	54,307	..	6,031	33,200	1,593	1,608
1932	1933–34	..	..	4,825	4,820	185	112
1933	1934–35	..	4,372	..	4,200	328	244
1934	1935–36	..	5,590	..	2,900	503	502
1935	1936–37	40,000	..	..	20,000	3,100	3,125
1936	1937–38	..	..	12,468	12,470	1,602	1,627*
1937	1938–39	3,000*	..	..	1,500*	..	..

*Figures not final.

SQUAWFISH

The early spring consumption of sockeye by squawfish 200 to 299 mm. long is shown in table VI for various years. Unfortunately the data are not strictly comparable throughout. In 1932 and 1933 the nets were left in the water (with rare exceptions) only one night; in 1936-38 they were commonly in for 2 to 3 nights, during which time some additional digestion would probably reduce the amount of recognizable food in the stomachs of the fish caught. Again, the greater survival rate of sockeye in 1936-38 as compared with 1932-33 means that only near actual migration time—April—should the sockeye be present in numbers proportional to the number of migrants, if years in these two different groups are compared.

Considering the 1932 and 1933 data first, there is an increase in frequency of occurrence and average number of sockeye, corresponding to a doubling of the sockeye population in those years (figure 1a and 1b). The average volume increased much less, since the 1933 migrants were individually only half as heavy as those of 1932. The years 1936, 1937 and 1938 also bear out the view that predation is proportional to abundance, though the numerical values are lower than in the earlier years, for reasons suggested above. These data are insufficient to establish this as an exact quantitative law, but do show that there is a definite correlation between number of sockeye present and the number consumed.

TABLE VI. Consumption of sockeye by squawfish 200-299 millimetres long, in the periods January and February and March and April of several years, and the corresponding seaward migrations of sockeye

	January and February				March and April				
Year	1932	1933	1937	1938	1932	1933	1936	1937	1938
Number of stomachs examined.	50	21	19	416	30	53	184	60	614
Number containing sockeye...	15	12	8	135	7	23	12*	22	139
Average volume of sockeye per stomach examined.....	0.57	0.72	0.83	0.78	0.27	0.42	0.61	1.51	0.74
Percentage by volume of total food.....	97	100	100	95	87	93	78	97	98
Average number per stomach examined.....	0.30	0.95	0.47	0.41	0.23	0.53	0.078	0.48	0.28
Average number per stomach having sockeye.....	1.0	1.7	1.1	1.3	1.0	1.2	1.1	1.3	1.2
Seaward migration (thousands)	789	1593	3100	1652	789	1593	503	3100	1652

*Since in 1936, a disproportionately large number of fish were taken in the latter part of April, this figure has been corrected to a representative value.

Char

The number of char stomachs examined was not large, so it is necessary to group them rather coarsely, by lengths and seasons, to smooth out fortuitous irregularities in sockeye consumption. In table VIIa a comparison is made of summer sockeye consumption with the estimated number of free-swimming fry entering the lake in the same spring. The numerous sources of error which affect both series of data are not sufficient to conceal a definite relationship between sockeye abundance and sockeye consumption. It is most apparent in the frequency of occurrence of the sockeye, shown graphically in figure 1g, where the points arrange themselves in a line with even greater regularity than could be anticipated. (Probably two sources of error compensate each other to produce this result: The greater survival rate of sockeye in 1935 to 1937 would mean that relatively more fry survived to summer time; hence they should be relatively better represented in the stomachs than in 1932-1934, only that in these earlier years the nets were visited more frequently, and fewer sockeye were lost by digestion after the char were caught.) The other measures of abundance of sockeye in the stomachs also fall fairly well into line with availability, excepting the last column tabulated.

Table VII—A. Fingerling sockeye salmon found in stomachs of char up to 499 mm. long, in July and August, compared with the estimated number of fry starting free-swimming life in each season.

Season	Estimated number of fry in May (thousands)	Number of stomachs examined	Number containing sockeye	Aver. vol. of sockeye per stom. examined	Percentage by volume of total food	Aver. no. per stom. examined	Aver. no. per stom. having sockeye
1932	33,200	21	17	5.3	64	6.4	11.0
1933	4,820	3	1	0.7	23	2.7	8.0
1934	4,200	32	6	0.7	13	0.5	2.5
1935	2,900	82	2	0.4	7	0.5	19.0
1936	20,000	19	10	1.4	13*	2.1	4.0
1937	12,470	30	9	1.0	59	3.0	10.0

*One fish of this group contained a large coho, comprising more than half of the total food of the group. Excluding it, the sockeye would comprise 30 per cent of the total food.

—B. Fingerling and yearling sockeye salmon found in stomachs of char up to 499 mm. long, from January to April, compared with the observed number of migrants in each season

Season	Number of migrants (thousands)	Number of stomachs examined	Number containing sockeye	Aver. vol. of sockeye per stom. examined	Percentage by volume of total food	Aver. no. per stom. examined	Aver. no per stom. having sockeye
1932	789	11	7	4.1	96	1.6	2.6
1933	1,593	6	5	7.1	88	5.3	6.4
1936	503	25	1	0.2	2	0.04	1.0
1937	3,100	18	5	2.1	60	0.7	2.4
1938	1,652	64	22	1.8	52	0.9	2.6

In table VIIb the number of migrants in various years is compared with sockeye consumption by char from January to April. In this case no readily apparent relationship between consumption and abundance is evident (figure 1c, 1d); though, if the years before and after 1934 be treated separately (as suggested above), there is some tendency for consumption to increase with abundance.

TROUT

Only from 1935 onward have trout been taken in numbers, so comparisons of their sockeye consumption with population available must be confined to that period. The best data pertain to the 300 to 399 mm. size group, and are presented in table VIII. The May-June figures are not particularly informative because of the small number of trout taken then which had eaten sockeye. However, in two years of small population (1935-36 and 1938-39) no sockeye at all were taken; while the 1936-37 stomachs showed a greater frequency of occurrence of sockeye than 1937-38, corresponding to a larger population. (The higher average number of sockeye in 1937-38 is due to two stomachs containing over 90 each).

The records of consumption from September to April show a rather consistent relationship with the populations presumed present (fig. 1, E.F.). The average frequency of occurrence of sockeye from September to April in 1936-37 is 0.65, in 1937-38 it is 0.35, the ratio being 2.0 to 1. The average number per stomach

TABLE VIII. Fingerling sockeye consumed by trout 300 to 399 millimetres long, in various years, for comparisons with populations present (see table V).

	May-June				September			October		
	35–36	36–37	37–38	38–39	35–36	36–37	37–38	35–36	36–37	37–38
Number of stomachs examined	32	72	48	26	47	27	8	20	41	9
Number containing sockeye	0	12	3	0	7	17	2	2	26	2
Average volume of sockeye per stomach examined	0	0.5	0.4	0	0.7	3.2	0.4	0.4	5.8	2.1
Percentage by volume of total food	0	14	25	0	34	82	20	34	93	46
Average number per stomach examined	0	2.2	3.8	0	0.55	1.78	0.38	0.10	2.12	1.33
Average number per stomach having sockeye	0	13.2	60.7	0	3.3	2.8	1.5	1.0	3.3	6.0

	December		January		February		March			April		
	36–37	37–38	36–37	37–38	36–37	37–38	35–36	36–37	37–38	35–36	36–37	37–38
Number of stomachs examined	38	89	12	35	7	65	36	36	52	65	41	47
Number containing sockeye	20	22	6	16	7	21	2	27	17	5	20	29
Average volume of sockeye per stomach	3.1	1.1	3.4	2.0	8.1	2.1	0.3	5.9	2.7	0.4	4.3	5.8
Percentage by volume of total food	100	87	100	94	100	92	16	99	90	26	92	91
Average number per stomach examined	1.42	0.51	1.10	0.77	3.00	0.88	0.08	1.80	0.86	0.14	1.46	1.81
Average number per stomach having sockeye	2.7	1.9	2.2	1.8	3.0	2.7	1.5	2.4	2.6	1.8	3.0	2.9

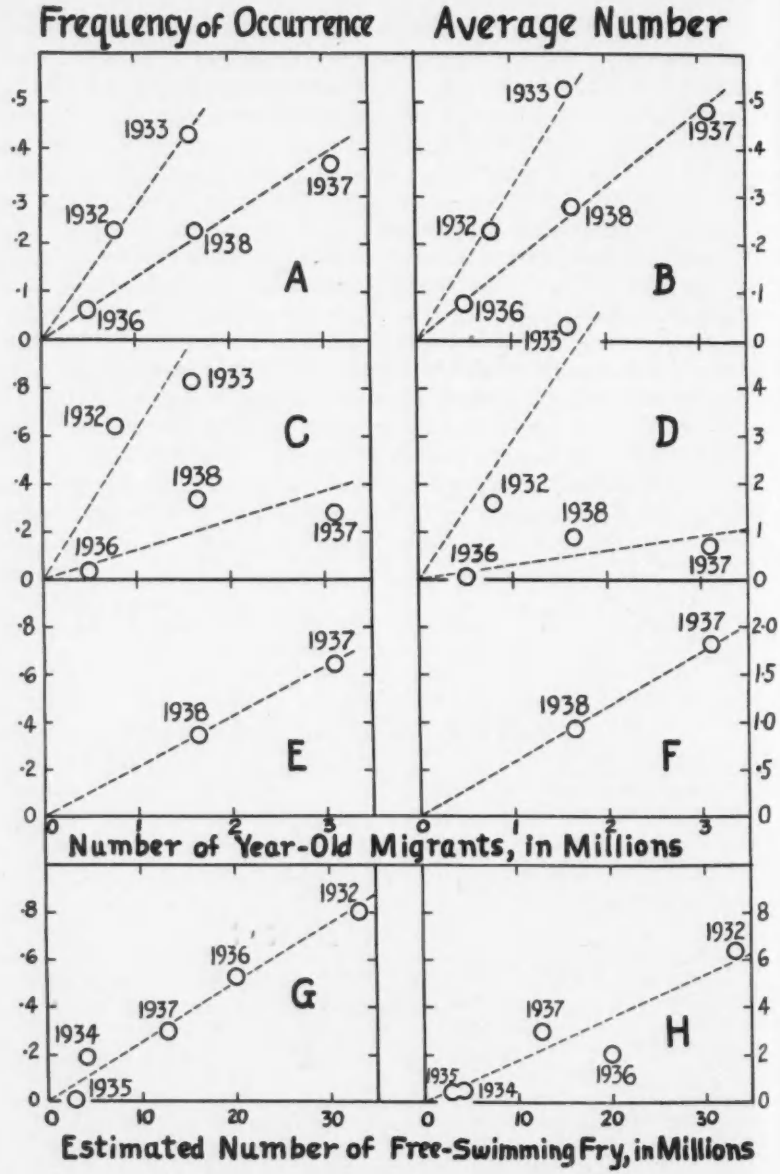

FIGURE 1. Frequency of occurrence and average number of sockeye in predator stomachs, compared to an estimate of the number of sockeye present. A and B: from stomachs of squawfish 200 to 299 mm. long, taken in March and April. C and D: from stomachs of char up to 499 mm. long, taken January to April. E and F: from stomachs of trout 200 to 299 mm. long, taken September to April (each month given the same weight). G and H: from char up to 499 mm. long, taken in July and August. All figures are based on the total number of stomachs examined, including empty ones. The dotted diagonal lines suggest the relationship if consumption were proportional to abundance of the sockeye.

examined is 1.81 in 1936-37, 0.93 in 1937-38, the ratio being 1.9:1.0; the ratio of the migrants in the two years is 3100:1652, or 1.9:1.0. Unfortunately the same comparison cannot be made throughout the whole season 1935-36; for September-October the frequency of occurrence of sockeye there is about 1/6 of 1936-37, for March-April about 1/15. (The ratio of migrations is 503:3100, or 1:6.2.) This latter fraction is one of several indications that the small populations may be less attacked, even in proportion to their numbers, than are the large ones, at least by some predators, or at certain seasons.

Coho

Tabulations similar to those for trout give much the same picture of sockeye consumption by coho, but are quantitatively much less reliable. For example, a comparison of the number found in April, per stomach examined, for the 200 to 299 size class, is as follows:

	Stomachs examined	Number with sockeye	Number of sockeye per stomach	Corresponding migration
1935-36	24	9	0.5	503
1936-37	3	2	2.3	3100
1937-38	45	24	1.6	1652

Other comparisons that can be made have the same general import.

"Buffer Foods"

The quantitative aspects of the action of predators upon the buffer organisms can be treated in much less detail than were the same for sockeye. However, there appears a general tendency toward a greater consumption of buffer foods when sockeye are less numerous, and vice versa. The trout and coho in March and April of 1936 consumed few sockeye as compared with 1937 and 1938, but a greater consumption of other organisms kept the total food per predator at about the same general level in the three years. Similarly, in squawfish taken in the autumn of 1935, sockeye comprised less than 5 per cent of the total food, as compared with 65 to 100 per cent in 1936 and 1937. If no other foods had been eaten, squawfish would have been practically starved in 1935; as it happened, other foods were taken in greatly augmented quantities, though they were not sufficient to make the average stomach-full in 1935 equal to that in 1936. July and August of 1934 and 1935 were characterized by a small consumption of sockeye by char, as compared with 1932 or 1937 when sockeye populations were much larger. What the regimen of the char lacked in salmon was, however, fully made up by other fish, chiefly *Cottus aleuticus* and *C. asper*, in the first-mentioned years. Coho are perhaps the only species which would suffer severely from a scarcity of sockeye, for their only considerable alternative food is insects, which during much of the year are not available.

It would appear therefore that in general the predators in the lake are able to tide themselves over years of scarcity of sockeye by eating other available foods in sufficient quantity to partly or completely compensate for the deficiency

of salmon. It may be enquired whether these alternative foods are present in greater numbers when sockeye are scarce, or whether the predators merely consume more of what is always at hand. Young sockeye being exclusively plankton-eaters, plankton is the only item of alternative food that might be directly released for predator consumption, and it of course is never very important in the food of the larger predators. An indirect effect is possible, in that other buffer organisms consume the plankton, notably sticklebacks, shiners and Aleutian sculpins; so these might conceivably increase in size or numbers with the decreased competition for food. None of these three, however, live in the pelagic habitat of the sockeye during summer, the season of rapid growth, so their opportunity to benefit by the scarcity of sockeye is limited. In any case the changes in sockeye numbers occur abruptly from one year to the next, and the change in consumption of buffer foods occurs equally abruptly, before the latter would have had time to multiply as a result of increased food. The increased consumption of buffer foods must be chiefly due, then, to the migration of predators to habitats where buffers are more common; or to their having a partial preference for sockeye, which makes the buffer species less subject to attack when the sockeye are at hand in good numbers.

From the standpoint of fisheries management, the presence of more or less adequate buffer species in the lake precludes the use of one possible method of controlling the abundance of predators in the lake. If sockeye were the only significant food of the latter, their numbers could readily be kept at a minimum by keeping the lake barren of sockeye during one of the four cycle years, thus leaving the populations in the other three relatively free from predation. As things are, no such procedure is feasible.

Discussion

The interaction between predators and their prey can take any one of several forms, depending on their relative abundance, and the nature of the physical and biotic environment. A survey of some of the more likely situations is presented below. A vertical division is made into situations (A) where predation is a matter of chance encounters between predator and prey—i.e. a kill occurs at each encounter, or at an invariable fraction of encounters; and (B) where the predators are always able to secure enough food, regardless of changes in the abundance of the prey within the limits under consideration. Under each vertical division four situations are cited; in 1. and 2. only one prey species exists, in 3. and 4. an alternative species of prey is available. The effects of a *decrease* in abundance of a given prey species is described in each case, as regards changes in (*a*) the catch of that species per individual predator, (*b*) the "rate of predation" upon the given prey species—i.e. the fraction of its total population killed per unit of time, and (*c*) the rate of capture, or fraction killed per unit of time, of the population of an alternative prey species. (Situations 3. and 4. may be combined with either 1. or 2., but are described in terms of 1. only—i.e. with the population uniformly vulnerable throughout the habitat.) In all cases abundance of predators is considered constant, as is the abundance of the alternative prey species, if present.

	A. Predation occurs at random encounters.	B. Predation is limited by predators' appetites
1. Prey is equally vulnerable throughout its habitat.	(*a*) Catch per predator decreases in direct proportion to abundance of prey. (*b*) Rate of predation is unchanged.	(*a*) Catch per predator is unchanged. (*b*) Rate of predation increases.
2. Prey is unequally vulnerable throughout its habitat, so that smaller populations are better protected.	(*a*) Catch per predator decreases more rapidly than abundance of prey. (*b*) Rate of predation decreases.	(*a*), (*b*) as under 1. above.
3. Alternative prey is available in the same habitat.	(*a*), (*b*) as under 1. above. (*c*) Rate of capture of alternative prey is unchanged.	(*a*) Catch per predator decreases. (*b*) Rate of predation increases but less rapidly than in case 1. above. (*c*) Rate of capture of alternative prey increases.
4. Alternative prey is available in a more or less different habitat.	(*a*) Catch per predator decreases more rapidly than abundance of prey. (*b*) Rate of predation decreases. (*c*) Rate of capture of alternative prey increases.	(*a*) Catch per predator decreases more rapidly than in case 3. above. (*b*) Rate of predation may increase or decrease; if the former, the increase is less than in case 3. (*c*) Rate of capture of alternative prey increases.

In comparing these possible situations with the data on predation of sockeye, there appears first a general tendency for catch per predator to be proportional to abundance of the sockeye population, more particularly when the population is of at least moderate size. This strongly suggests that predation is principally of the strictly random type (A1), and that modifying factors (A2, A3, A4) are of little importance. This is in good agreement with the finding of Foerster (1938) that the survival rate of young sockeye during their lacustrine life is not correlated with the size of their population.

A decrease in catch per predator of greater magnitude than would be proportional to population is suggested in a few instances in the data. These instances suggest that at times alternative prey may have led predators to other habitats to some extent (A4), or less probably, that a smaller sockeye population is on the whole in a more secure situation in the lake (A2).

It was pointed out earlier that there is a marked increase in the consumption of other foods by predators, in years when sockeye are scarce, and that there is reason to suppose these other foods do not increase much in abundance immediately the sockeye become scarce. From the schedule above it appears that this increased consumption would occur, among the A-type situations, only if the alternative foods were most common in parts of the lake not so much frequented by

sockeye, so that in pursuing the former the predators would afford the latter some relief from predation. Another possibility exists, however, and should be seriously considered, since the amount of relief from predation which the smaller sockeye populations receive does not appear to be great. One of the principal alternative prey species is the stickleback, which on account of its spines may be imagined to be in disfavour with predators, and consumed by them only under straitened circumstances. (It is rarely taken by the smaller trout, though of much smaller size than sockeye which they eat regularly.) In this event there could occur an increase in consumption of the alternative food, in the same habitat, which would of course not benefit the sockeye in any way.

That predation upon sockeye in Cultus lake has ever been limited by the appetites of the predators, does not appear in these data. The most comprehensive series of data available (table VIII) shows that the frequency of occurrence and average number of sockeye found in trout were in direct proportion to population, comparing the largest population on record with one half as large. Further, with these large populations the number of empty predator stomachs found is at most seasons still considerable, and in the case of squawfish is very large—which would hardly be the case if there were more than sufficient opportunity for the fish to fill them. We may then ignore most of the predation situations of series B above. Certainly none occur of types B1 or B2, in which catch of sockeye per predator is constant at all population levels. Situations B3 and B4, which in their effects somewhat resemble A4, are perhaps possible in exceptional circumstances, especially with trout and coho at seasons of the year when food is unusually abundant, as notably in April.

It would be a mistake to apply the information obtained concerning predation on sockeye to fish predation generally, though there may be other species with similar predation patterns. In the case of the sockeye, a pelagic habitat in which there is a minimum of protection for fish (other than that provided by their colour and physical powers) would appear to make random predation the logical type to be expected. Relief from predation comes only when this habitat becomes so destitute of prey of all kinds that the predators begin to leave for better feeding areas. However, many fishes living in non-pelagic habitats, either in rivers or near the bottom of a lake where logs and vegetation offer protection, may reasonably be supposed to present a different picture. In their case, the average vulnerability of a prey species will probably tend to increase with population abundance, at least above a certain minimum, as the protected situations become filled up and the surplus fish must forage in more exposed waters. Then, if hungry predators are continuously at hand, the predation situation will be of type A2, often combined with A3 or A4. Such a situation has been found to exist in regard to winter predation upon certain birds, notably the Iowa bobwhite (Errington and Hamerstrom 1936).

SUMMARY

Young sockeye salmon are attacked by predatory fish in Cultus lake from the time they rise from their nests until they leave the lake a year later. Trout, coho salmon and char are active against them throughout the year; squawfish

consume them from late September to April, but eat other foods in summer; prickly sculpins take newly-emerged fry, and possibly fingerlings near time of migration.

Sockeye are in most years an important food of these predators, and when abundant may constitute practically their only food at certain seasons.

Consumption of sockeye, as indicated by frequency of occurrence and average number found per stomach, appears in general to be proportional to their abundance. There are, however, some indications that sockeye of smaller populations may be attacked less often than in proportion to their numbers.

In years of small sockeye populations, consumption of alternative foods by predators increases, though there is little reason to suppose that most of these food organisms should increase in numbers because of the scarcity of sockeye. It appears that predators must to some extent move to habitats where buffer foods are more common, and they may also have a scale of food preferences in which sockeye rank high, so that some buffer species are avoided as long as sockeye are abundant.

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REFERENCES

ERRINGTON, PAUL L., AND F. N. HAMERSTROM. *Iowa Agr. Expt. Sta. Res. Bull.* **201**, 1-443, 1936.

FOERSTER, R. E. *Contr. Canad. Biol.* N.S. **2** (2), 337-442, 1925.
Contr. Canad. Biol. Fish. **5** (3), 57-82, 1929.
Copeia **1930** (3), 90, 1930.
Trans. Amer. Fish. Soc. **64** (1934), 379-381, 1935.
Bull. Biol. Bd. Can. **53**, 1-16, 1936.
J. Fish. Res. Bd. Can., **4** (3), 151-161, 1938.

FOERSTER, R. E., AND W. E. RICKER. *Verh. int. Verein. Limnol.*, **8** (3), 151-167, 1938.
J. Fish. Res. Bd. Can., **5** (4), 313-335, 1941.

RICKER, W. E. *Biol. Bd. Can. Prog. Rep. Pac.*, **18**, 3-4, 1933.
J. Biol. Bd. Can., **3** (5), 450-468, 1937.

ROBERTSON, A. *Trans. Amer. Fish. Soc.* **66** (1936), 279-282, 1937.

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